Cardiovascular Diabetology

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A glucose time in range of 70% attenuates the senescence-inducing and pro-inflammatory effects of hyperglycemia

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Abstract

Background The Time In Range (TIR) represents the amount of time spent by a given individual in the range close to normoglycemia, *i.e.* 70–180 mg/dl. On the basis of studies demonstrating an association of TIR with the incidence of diabetes complications, guidelines recommend a target of at least 70% of TIR for most people with diabetes. However, no study has explored the effect of variable degrees of TIR on molecular mechanisms relevant for the development of diabetes complications.

Methods We exposed endothelial cells and monocytes to increasing percentages of TIR, *i.e.* 50%, 70%, 85% by changing cell media twice a day as appropriate, as well as to constant normoglycemia (*i.e.* fixed 100 mg/dl of glucose for endothelial cells) and hyperglycemia (*i.e.* 500 mg/dl glucose), evaluating the development of senescence, of the associated pro-inflammatory response, and monocytes adhesion to endothelial cells as a functional assay. We then assessed the expression of a plethora of markers of senescence and inflammation at the mRNA level in peripheral blood mononuclear cells (PBMC)s derived from individuals with early (*i.e.* 1-year post-diagnosis) type 1 diabetes (T1D, n = 37), categorized according to the TIR (< or > 70%) observed in the previous 14 days, comparing the two groups through ANCOVA adjusted for HbA1c. As a confirmatory analysis, we also compared the expression of the same markers in people with Time Above Range (TAR), considered as the whole time above 180 mg/dl, ≥ vs < 30%. Correlations between TIR values and the expression of the same markers were tested through linear regression.

Results Constant hyperglycemia promoted the development of senescence in endothelial cells and induced inflammatory responses in both endothelial cells and monocytes, promoting also monocytes adhesion to endothelial cells. A TIR of 70%, but not of 50%, suppressed these effects while a TIR of 85% did not provide additional benefit. Data from people with T1D mirrored such results, as demonstrated by the higher expression of p16, a marker of senescence, and of IL-6, MCP-1, and CXCL1, three inflammatory mediators, in PBMCs from individuals with TIR < 70% and compared with those with TIR > 70%, independently of HbA1c. Similar results were obtained when comparing

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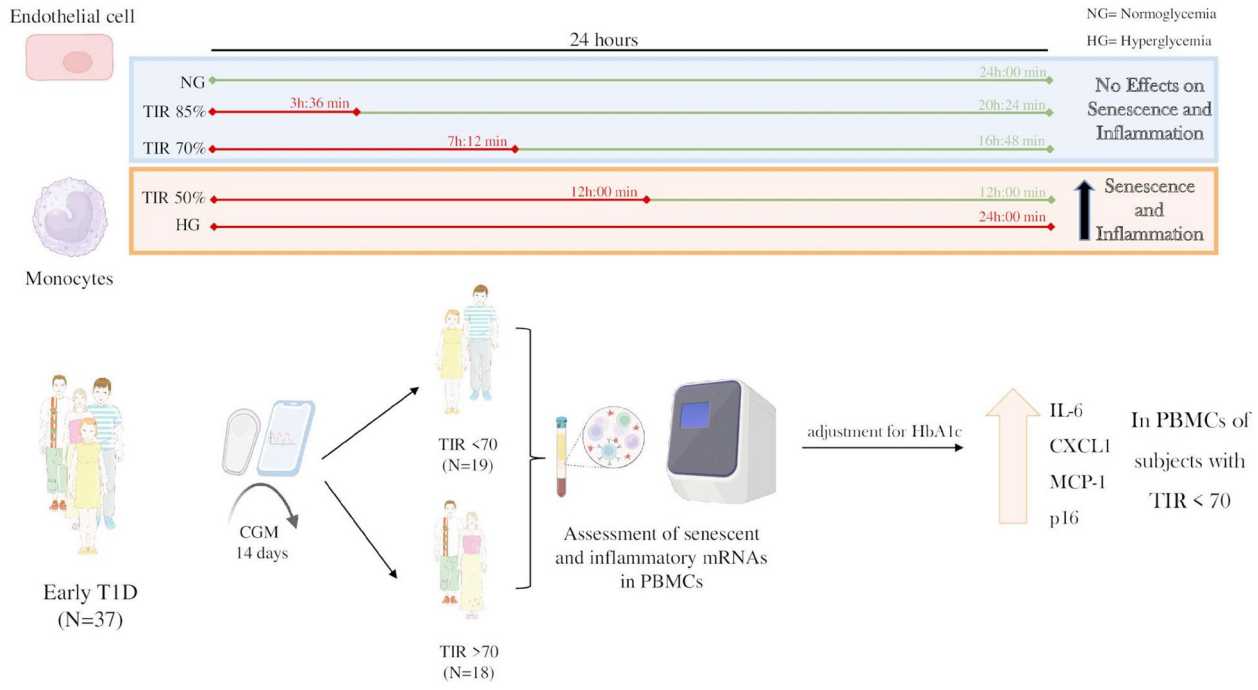
people with TAR ≥ vs < 30%. When considered as a continuous variable, TIR values were correlated with p16, IL-6, and CXCL1.

Conclusions A TIR above 70% is associated with attenuated pro-senescence and pro-inflammatory effects of hyperglycemia. These molecular results support the TIR target currently recommended by guidelines, especially for people with T1D.

Keywords Continuous glucose monitoring, TIR, TAR, Hyperglycemia, Inflammation, Senescence, Endothelial cells, Monocytes, Type 1 diabetes, PBMC

Graphical Abstract



Research insights

What is currently known about this topic?

- On the bases of observational studies and data from post-hoc analyses of trials, guidelines recommend a target of more than 70% of Time In Range (TIR) for most people with diabetes. However, no study has explored the effect of different degrees of TIR on molecular mechanisms relevant for the development of diabetes complications.

What is the key research question?

- Which degree of TIR is needed to halt the pro-inflammatory and pro-senescence effects of hyperglycemia in endothelial cells and monocytes? Are these molecular effects mirrored in PBMCs from people with early T1D and discordant for TIR?

What is new?

- A TIR of 70%, but not of 50%, suppressed the pro-inflammatory and pro-senescence effects of hyperglycemia, while a TIR of 85% did not provide additional benefit. PBMCs from people with a TIR < 70%, compared with those >70%, showed a higher expression of senescence and inflammatory markers, also after adjustment for HbA1c.

How might this study influence clinical practice?

- These molecular results support the TIR target currently recommended by guidelines.

Introduction

A number of metrics of glycemic control juxtaposes glycated hemoglobin (HbA1c) in the management of people with diabetes [1, 2]. Among others, selected measures of glycemic control are provided by continuous glucose monitoring (CGM) devices, which continuously measure interstitial fluid blood glucose levels. One of these

metrics, the Time In Range (TIR), represents the amount of time spent by a given individual in the range close to normoglycemia, *i.e.* 70–180 mg/dl of glucose [1, 2].

Guidelines recommend a target of at least 70% of TIR for most people with diabetes [1]. This class B recommendation relies on a reasonable number of observational studies and post-hoc analyses of data derived from clinical trials suggesting an association between TIR and both the prevalence and the incidence of multiple diabetes complications [2, 3]. On the other hand, preliminary findings suggest that increasing TIR up to 85% might be beneficial in terms of a reduced incidence of mortality [4]. Given the paucity of conclusive clinical evidences, molecular studies could eventually help to understand if increasing the percentage of TIR could be beneficial at least in terms of attenuating the deleterious pathways instigated by hyperglycemia. However, no study has explored the effect of different degrees of TIR on molecular mechanisms relevant to the development of cardiovascular diseases or the microvascular complications of diabetes.

To this aim, we tested the effect of variables degrees of TIR, *i.e.* 50%, 70%, and 85%, and compared with constant normoglycaemia or constant hyperglycemia, on the development of senescence and inflammation, two of the molecular phenomena held to contribute to the development of diabetes complications [5–7], in endothelial cells and monocytes exposed to these conditions for 10 or 5 days, respectively. To mimic different degrees of TIR, media was changed twice a day, as appropriate, using two fixed levels of glucose in cell media. Then, to gain preliminary insights into the human relevance of such findings, we assessed the expression of senescence and inflammatory markers in Peripheral Blood Mononuclear Cells (PBMC)s from people with recently developed type 1 diabetes (T1D), *i.e.* one year after diagnosis, categorized according to the TIR observed in the 14 days preceding samples collection, *i.e.* above or below 70%, adjusting the analyses for HbA1c.

Materials and methods

Cell cultures, treatments, and readouts

Human umbilical vein endothelial cells (HUVEC) from pooled donors (Clonetics, Lonza) were cultivated in endothelial basal medium (EBM-2, CC-3156, Lonza) supplemented with SingleQuot Bullet Kit (CC-4176, Lonza). The development of cellular senescence was verified through senescence-associated (SA)-Beta-Gal staining (#9860, Cell Signaling). Human monocytes THP-1 cells (ATCC) were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% l-glutamine (all from Euroclone).

Young endothelial cells *(i.e.*, SA-Beta Gal < 10%) were seeded at a density of 5000/cm^2 and exposed to increasing percentages of TIR (50%, 70%, and 85%), as well as to constant normoglycaemia (a fixed level of 100 mg/dl glucose) or constant hyperglycemia (fixed 500 mg/dl glucose), for ten days with daily media change. To obtain the desired degrees of TIR, media was changed twice a day at appropriate timing (as depicted in the study design of Fig. 1A). For instance, to obtain a TIR of 50%, cells spent 12 h a day at 100 mg/dl of glucose and the other 12 h at 500 mg/dl. Glycemia was not fluctuating neither during normoglycemia nor during hyperglycemia. The same degrees of normoglycemia and of hyperglycemia (500 mg/dl glucose), commonly used in molecular experiments [8], were applied to all the TIR conditions, in order to isolate the effect of TIR from those related to the magnitude of glycemic excursions. The same design was applied to monocytes for 5 days, adjusting the relative amount of glucose to 200 mg/dl for normoglycemia and to 600 mg/dl for hyperglycemia, a common approach with this cell type [8, 9].

Molecular readouts were markers of senescence and of the associated inflammatory response assessed through kit, *i.e.* for SA-Beta-Gal, or through either RT-PCR or Western Blot for the other variables, as described in the subsequent sections. According to existing literature [5–7], we used SA-Beta-Gal, p16, p21, and PAI-1 as markers of senescence while IL-1α, IL-1β, IL-6, IL-8, TNFα, CXCL1, MCP-1, and NLRP3 were selected as relevant inflammatory markers.

RNA extraction and RT qPCR

Total RNA was extracted using the RNA Purification Kit (37,500, Norgen Biotek) and assessed for concentration and purity with a NanoDrop spectrophotometer (Thermo Fisher). Samples with a 260/280 ratio of approximately 2.0 were selected for further analysis. One microgram of RNA was reverse-transcribed using the Superscript III Reverse Transcriptase Kit (Invitrogen) following the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was conducted on a QuantStudio 6 Flex detection system (Applied Biosystems) with TB Green® Premix Ex Taq™ reagents (RR420A, Takara Bio). The thermal cycling conditions followed previously published protocols [8] and the primers used are listed in Supplementary Table 1. GAPDH was used as the reference gene for normalization for in vitro experiments while β-Actin was used to normalize human PBMCs data [10].

Western blot

Cells were lysed in RIPA buffer (R0278, Merck) with 1% protease inhibitor (11,873,580,001 cOmplete™, EDTA-free Protease Inhibitor Cocktail, Merck), 1% phosphatase inhibitor II and III (p5726, p0044, Merck). Protein

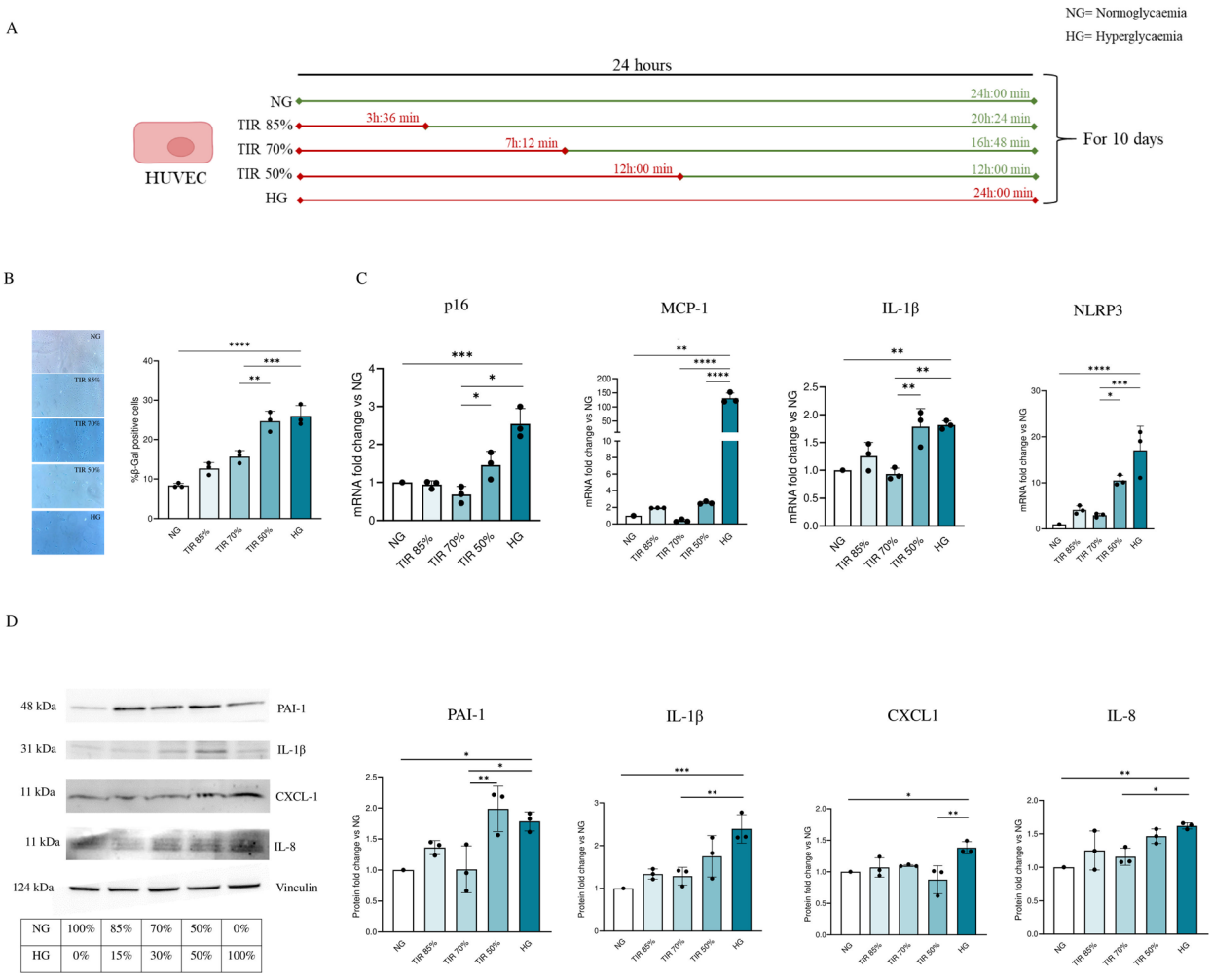


Fig. 1 Effect of variables degrees of TIR on endothelial senescence and inflammation. **A** Graphical summary of the experimental conditions, **B** representative SA-βGal staining, along with the relative quantitation, of endothelial cells exposed to increasing degrees of TIR (50%, 70%, and 85%), as well as to constant normoglycemia (NG) or hyperglycemia (HG), **C** mRNA expression levels of p16, MCP-1, IL-1β, and NLRP3 assessed through RT-PCR and presented as fold changes over NG, and **D** representative Western Blots of PAI-1, IL-1β, CXCL1, and IL-8, along with their quantitation through densitometric analyses, presented as fold changes over NG and normalized using Vinculin as internal control, in the same experimental settings. Data are mean ± SD of three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. For all comparison, ANOVA followed by Tukey test was used

concentration was determined using the Bradford assay (23,200, Thermo Fisher). For Western blots, 50 µg of lysate was separated by electrophoresis using PAGE gels (NP0335BOX, NuPAGE™ Bis–Tris Mini Protein Gels, 4–12%, Thermo Fisher) and transferred to nitrocellulose membranes (GE10600020, 0.45 µm, Merck). After blocking with 5% non-fat dried milk (70,166-500G, Merck) or 5% bovine serum albumin (A1470-100G, Merck), membranes were incubated overnight at 4 °C with the following primary antibodies: anti-IL-1β (ab254360, Abcam), anti-IL-6 (#12,153; Cell Signalling), anti-IL-8 (ab110727; Abcam), anti-CXCL1 (#24,376; Cell Signalling), anti-TNFα (#6945; Cell Signalling), anti-PAI-1 (#11,907; Cell Signalling), and anti-Vinculin (#13,901; Cell Signalling) all diluted 1:1000, Secondary IgG HP-conjugated anti-rabbit HRP-linked antibody (#7074; Cell Signalling, 1:3000) were applied for 1 h at room temperature. Immunoreactive proteins were revealed with WESTAR ETA ULTRA C 2.0 (XLS075,0100, Cyanagen) using UVITEC Alliance Q9. Vinculin was used as the loading control. Densitometric analysis was performed with UVITEC Nine Alliance.

Monocytes adhesion to endothelial cells

HUVEC were plated as a monolayer and exposed to increasing percentages of TIR, NG and HG for 10 days as described above. At the end of the treatment, we tested monocytes adhesion using The Vybrant™ Cell Adhesion Assay Kit (V-13181, Invitrogen). Five × 10^6 THP-1 monocytes were labeled with 7.5 µM of calcein-acetoxymethyl ester (AM) at 37 °C for 30 min. After washing cells two times with RPMI, the labelled suspension (5 × 10^5 cells)

was added to HUVEC previously exposed to increasing percentages of TIR, NG and HG for 10 days. After an incubation for 3 h at 37 °C, nonadherent, calcein-labeled cells were removed by careful washing with RPMI. The fluorescence of adherent calcein AM labeled THP-1 cells was measured using the spectrophotometer Synergy HT (Biotek), setting an excitation of 485 nm. Absorbance of control cells (NG) was set at 1 and the others calculated as relative fold changes.

Sample size calculation for the cohort study

Given the lack of previous studies using such an approach, we estimated a sample size based on the observations obtained from our preliminary in vitro experiments. Using p16 as the reference, *i.e.* the most commonly used marker of senescence [5–7], we observed a mean difference between treatments above vs below 70% of TIR of 0.5 arbitrary units (*i.e.* the effect size) with a standard deviations of 0.5 units. Considering a Beta of 0.2, an alpha of 0.05, and an expected equal proportion of subjects in the two groups, with the intention of comparing the two means through T statistic it results that two groups of 17 people each (total n = 34) are needed to observe a difference in the expression of this marker. We estimated that such sample size would suffice also for the comparison of the other markers, which showed similar data in preliminary experiments.

Study cohort, PBMCs collection, and endpoints

Participants were selected from a National Childhood Registry for type 1 diabetes and were regularly managed at the Department of Endocrinology, Diabetes, and Metabolic Diseases at the UMC Ljubljana University Children Hospital, Slovenia. The participants enrolled provided their written informed consent and the protocol was approved by the Slovenian Ethical Committee (nr. *0120–6/2023/3*).

Between March 2023 and March 2025, participants were recruited during a routine follow-up visit approximately one year after type 1 diabetes diagnosis according to selected study inclusion criteria: the use of CGM devices, had available clinical data and complete CGM data relative to the previous 14 days, and were on therapy only with insulin (any treatment modality, including automatic insulin delivery systems). Main exclusion criteria were a diagnosis of diabetes other than T1D, use of glucose-lowering drugs beyond insulin, incomplete CGM data, lack of clinical information, and inability to provide appropriate volumes of blood samples to isolate PBMCs. People with recently developed disease were selected for participation in order to minimize the burden of comorbidities thus maximizing the chances of isolating the effect of glycemia on inflammatory and senescence variables [5–7], as well as to increase the likelihood of creating two balanced groups discordant only for glucose control.

Clinical examinations were conducted and electronic health records were evaluated to collect demographic, clinical, and therapeutic data. Subjects were all Caucasian and pubertal status was defined as previously suggested [11]. During a regular clinical visit, CGM data collected and blood samples were obtained according to standard procedures after overnight fasting for analysis of biochemical variables and to isolate PBMCs, which were isolated starting from fresh heparin blood tubes through Ficoll-Paque using a standard protocol [12]. Considering CGM data relative to the 14 days preceding blood withdrawal, people were assigned to one of two groups: individuals with TIR < 70% or subjects with TIR > 70%. The primary endpoint of the study was the expression of p16 and other common markers of senescence and inflammation, *i.e.* p21, IL-1α, IL-1β, IL-6, IL-8, CXCL1, MCP-1, and NLRP3 [5–7], in PBMCs among people with TIR < 70% and those with TIR > 70%, assessed at the mRNA level. The correlations between TIR values, considered as continuous variables, and the same markers were exploratory endpoints. As an additional exploratory analysis, we also compared the expression of the same markers in the same population categorized according to the Time Above Range (TAR), considered as time above 180 mg/dl without the further stratification commonly used in clinical practice differentiating the 180–250 mg/dl from the > 250 mg/dl range [1–3]. Such an approach was chosen to avoid creating too much groups, given the limited sample size. Considering this categorization, we opted to set the threshold for categorization as a TAR < or ≥ 30%, which is the sum of the targets suggested by guidelines which recommend a TAR < 25% for the 180–250 mg/dl glycemia range and < 5% for the > 250 mg/dl glucose range [1–3].

Statistical analysis

For in vitro experiments, at least three different batches were used for endothelial cells while the same number of technical replicates were done for monocytes. Levels of different mRNAs or proteins among different treatments were compared using ANOVA followed by Tukey test.

For the cohort study, the distribution of variables was assessed with the use of the Shapiro–Wilk test. Clinical characteristics were compared between the two groups with a t-test for normally distributed data, the Mann–Whitney U test for non-normally distributed data, and Fisher's exact test for categorical variables. Since, as expected, HbA1c was different among the groups of people with TIR < 70% vs those with TIR > 70%, and considering that this variable is held to affect senescence and inflammation [5–7], the comparison of the expression of p16, p21, IL-1α, IL-1β, IL-6, IL-8, CXCL1,

MCP-1, and NLRP3 in the two groups, *i.e.* the primary endpoint, was performed using ANCOVA adjusted for HbA1c. The same approach was used to compare the expression of the same markers in the groups of people with TAR ≥ or < 30%. Linear regression analyses were performed to estimate the association of TIR values, considered as a continuous variable, and the same markers. A two-sided P value of less than 0.05 was considered significant. Figures were prepared with the use of GraphPad Prism, version 9.1.2, which was used also for statistical analysis with the exception of the ANCOVA test, which was performed with StatsCalculators [13].

Results

To explore the effect of variables degrees of TIR on senescence and inflammation, we treated endothelial cells with increasing degrees of TIR for 10 days, and compared these conditions with constant hyperglycemia or normoglycemia (Fig. 1A). Constant hyperglycemia induced senescence and its associated pro-inflammatory phenotype in endothelial cells, as demonstrated by the increased percentage of SA-Beta-Gal positive cells (Fig. 1B), the higher abundance of p16, p21, IL-1β, and NLPR3 mRNAs (Fig. 1C), and by the increased expression of IL-8, PAI-1, IL-1β, and CXCL1 (Fig. 1D) at the protein level. Of note, while 50% TIR only partially attenuated these effects, a TIR of 70% was associated with suppressed pro-senescence and pro-inflammatory effects of hyperglycemia, with no additional benefit provided by a TIR of 85% (Fig. 1B–D). To corroborate these data, we also tested the adhesion of monocytes to endothelial cells as a functional readout. At the end of the 10 days of treatment with the different conditions, an increased adhesion was observed in cells exposed to constant hyperglycemia and to 50% TIR, but not in the other conditions (Supplementary Fig. 1), a finding in line with inflammatory genes expression data.

A similar pattern of inflammation was observed in monocytes exposed to the same conditions for 5 days. Indeed, constant hyperglycemia increased the expression of IL-1α, IL-1β, TNFα, and MCP-1 mRNAs and of IL-1β, IL-6, IL-8, TNFα, and CXCL1 at the protein level (Supplementary Fig. 2). Most of these markers were not increased when cells were exposed to 70% TIR but they were still higher than normoglycemia with a TIR of 50% (Supplementary Fig. 2), suggesting that a TIR of 70% is both needed and sufficient to ameliorate the pro-inflammatory effects of hyperglycemia on monocytes.

To gain preliminary insights about the human relevance of such findings, we explored the expression of a range of senescence and inflammation-related mRNAs, *i.e.* p16, p21, IL-1α, IL-1β, IL-6, IL-8, CXCL1, MCP-1, andNLRP3, in PBMCs derived from a cohort of people with recently developed T1D and categorized accordingly to the TIR observed in the 14 days preceding blood collection (Fig. 2A). The flowchart summarizing patients' inclusion/exclusion is reported in Fig. 2B. Twenty-one individuals were excluded due to the insufficient amount of blood to isolate PBMCs while the others could not be included since they did not use CGM devices or has inadequate data (Fig. 2B). The resulting two groups (n = 37) of people with TIR > 70% vs individuals with TIR < 70% were comparable for the main clinical characteristics known to affect senescence and inflammation [5–7] with the exception of HbA1c (Table 1), which was thus used as a covariate to adjust the analyses. People with a TIR < 70%, compared with those with TIR > 70%, showed a higher expression of IL-6, CXCL1, MCP-1, and p16 (Fig. 2C), as assessed by ANCOVA adjusted for HbA1c, but not of p21, IL-1α, IL-1β, IL-8, and NLRP3 (Supplementary Fig. 3). When considered as a continuous variable, TIR values had a significant correlation with the levels of IL-6, CXCL1, and p16, as evidenced by linear regression analyses (Supplementary Fig. 4). As an additional analysis, we also compared the expression of the same inflammatory markers categorizing the individuals according to the TAR measured during the same period, dividing the population according to having a TAR (including both the ranges of 180–250 mg/dl and > 250 mg/dl glycemia) ≥ or < 30% (clinical characteristics presented in Supplementary Table 2). Consistently, people with TAR ≥ 30%, compared with those below such threshold, had a higher expression of IL-6, CXCL1, MCP-1, and p16 (Supplementary Fig. 5).

Discussion

Existing guidelines recommend a TIR > 70% for most people with diabetes, a class B recommendation relying on clinical data mostly derived from observational studies [1–3]. However, no molecular study demonstrate that such threshold avoids the noxious effects of hyperglycemia. Here we showed for the first time that a TIR of 70% is associated with a suppression of the effects of hyperglycemia on senescence and inflammation in endothelial cells and monocytes, a result corroborated by the observation that the expression of a set of related markers are higher in PBMCs of people with early T1D and a TIR < 70%, compared with individuals with the same condition but having a TIR > 70%, a result observed also after adjustment for HbA1c. As a confirmatory analysis, we also observed that people with a TAR ≥ 30% had a higher expression of these markers compared with individuals with TAR < 30%. Overall, these data suggest that hyperglycemia reduced to 30% per day appears to suppress the pro-senescence and pro-inflammation effects of high glucose.

Our approach has inherent limitations. Indeed, to try to isolate the effect of time, we only tested the effect of

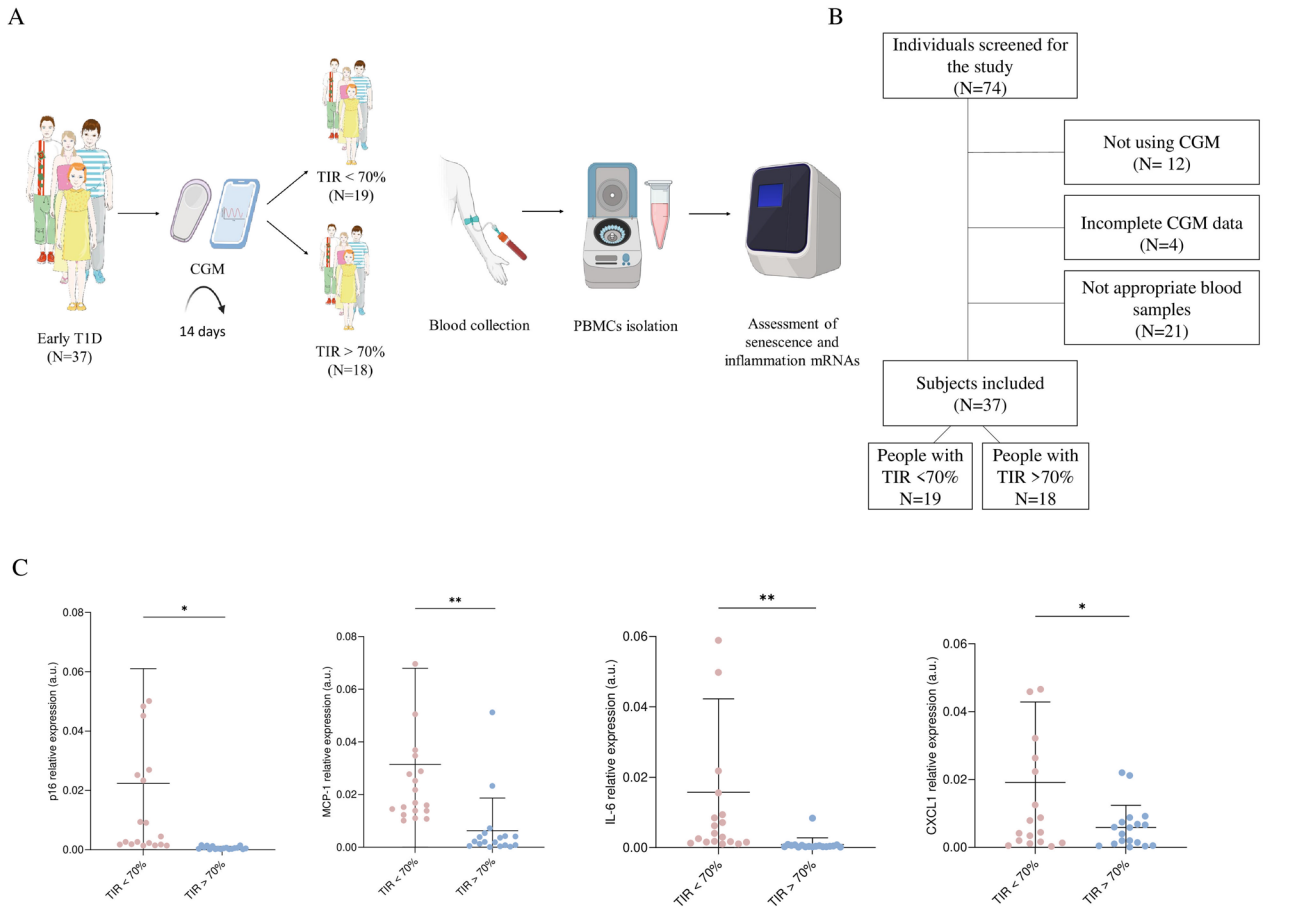


Fig. 2 Expression of senescence and inflammatory markers in PBMCs from people with T1D (n = 37). **A** Experimental design of the study, **B** Flowchart of included/excluded individuals, **C** mRNAs expression levels of p16, MCP-1, IL-6, and CXCL1, normalized to β-Actin and presented as relative expression, in PBMCs from people with T1D and a TIR < or > 70% in the previous 14 days. Data are mean ± SD and individual points are shown. *$p < 0.05$; **$p < 0.01$. For all comparison, ANCOVA adjusted for HbA1c values was used

one level of hyperglycemia, *i.e.* 500 mg/dl. This level of glucose, commonly used in molecular experiments [5–9], is hardly observed in people with diabetes after disease discovery and treatment. Thus, it is unknown whether such results extend to other levels of glycemia more commonly observed in real life. Similarly, for normoglycemia we only used the standard condition for cell culture, *i.e.* 100 mg/dl for endothelial cells and the double of such concentration for monocytes. However, recent data suggest that even small changes in glycemia can be accompanied by a differential effect on complications in diabetes [14] and a metric named Time In Tight Range (TTIR) was recently introduced to monitor the time spent by an individual in the range of glucose between 100 and 140 mg/dl [15]. Whether glycemia levels set as upper TIR or TTIR margins have a different effect on senescence and inflammation when compared to standard normoglycemia is unknown, an aspect warranting future investigation. The same applies to other levels of hyperglycemia more closely resembling those observed in people with diabetes. To this regard, it is important to emphasize that, to try to isolate the effect of time, here we used fixed levels of glucose, varying the relative exposure to either 100 or 500 mg/dl by changing cell media twice a day as appropriate to reproduce the percentages of TIR. Thus, levels of glucose were not fluctuating within the TIR range and this are not representative of the scenario observed in blood of people with diabetes. Given that the magnitude of glucose oscillations are known to influence a range of pathways including inflammation [16–18], these aspects might have influenced the results and represent a limitation of this study. Future work should explore the effect of multiple glucose levels better mimicking real-world glycemic variability. More broadly, upcoming studies should explore whether CGM data could be analyzed by a prediction algorithm to reflect more longitudinal outcomes [19] and not just short-term, intermediate endpoints.

The pathways studied do not encompass all the molecular alterations induced by hyperglycemia [20]. Thus, we cannot exclude that studying other molecular phenomena or mechanisms may provide different results. Given the impossibility to assess all the imbalances induced by

Table 1 Clinical characteristics of included participants

Variable	TIR < 70 (n = 19)	TIR > 70 (n = 18)	*p*-value
Age (years)	13.4 (± 2.6)	13.2 (± 3.5)	0.884
Sex, male, n (%)	13 (68.4)	15 (83.3)	0.447
Prepubertal, n (%)	3 (15.8)	5 (27.8)	0.447
Pubertal, n (%)	11 (57.9)	8 (44.4)	0.517
Postpubertal, n (%)	5 (26.,3)	5 (27.8)	> 0.999
HbA1c (%)	7.35 (6.9–8.3)	6.4 (6.1–6.7)	**< 0.001**
BMI (kg/m^2)	18.1 (15.8–23.1)	19.2 (16.8–22.8)	0.707
Systolic blood pressure (mmHg)	109 (106–120)	109 (103–114.3)	0.625
Diastolic blood pressure (mmHg)	66.4 (± 7.8)	62.1 (± 8.6)	0.117
Total cholesterol (mg/dl)	166.5 (46.2)	149.7 (37.9)	0.238
LDL cholesterol (mg/dl)	106.8 (48.8)	94.5 (18.7)	0.323
HDL cholesterol (mg/dl)	54.1 (46.4–69.7)	58 (54.1–65.7)	0.759
Triglycerides (mg/dl)	53.1 (39.9–70.9)	70.9 (53.1–106.3)	0.086
Celiac disease autoantibodies (TTG) -tTG IgA	2.3 (1.7–3.3)	1.95 (1.5–2.3)	0.429
Thyroid autoantibodies (S-anti TG)	2.4 (1.5–36.3)	6.4 (5.4–17.7)	0.302
Thyroid autoantibodies S-anti TPO	39 (35.5–53)	36 (31.3–105)	0.585

Data are presented as mean ± SD, median (Q1-Q3), or n (%) according to the type of variable and its distribution. Significant *p* values are highlighted in bold

hyperglycemia in one study, we opted for senescence and inflammation on the basis of their emerging key role for the development of complications [5–7, 21–23]. However, other alterations induced by hyperglycemia, *e.g.* oxidative stress and autophagy, might eventually benefit of a higher TIR or demonstrate no alteration with lower degrees of TIR. Future studies might help to clarify this aspect. In addition, given the study design relying on CGM data, here we only considered the effect of short-term glucose changes on inflammation and senescence [24]. Of note, also long-term glucose variability, *e.g.* the visit-to-visit oscillations of HbA1c, are known to influence these two biological phenomena [25, 26] and, more broadly, has been consistently associated with the development of diabetes complications also independently of mean glycemic control [27, 28]. To understand the relative contribution of short- and long-term variability, future studies should explore the impact of visit-to-visit HbA1c variability on immune and endothelial cells inflammation in comparison with measures of short-term glucose variability.

Another key consideration is that we selected a cohort with recent onset T1D to try to isolate as much as possible the effect of glycemia/TIR on senescence and inflammation and avoid the confounding effect of other metabolic or anthropometric variables associated with an alteration of these mechanisms and related pathways, *e.g.* age, obesity, and lipids [5–7, 21–23]. However, such an approach impedes to extend the findings to people with type 2 diabetes (T2D) or other populations with older age and/or other metabolic conditions.

From a general perspective, molecular data cannot be used to provide clinical recommendations. However, they might suggest whether it's worth designing specific studies assessing the effect of different targets of TIR on the incidence of diabetes complications. Our results do not support the idea that setting TIR targets above 70% could provide an additional benefit, at least at the molecular level. They also corroborate the idea that a 50% TIR is not sufficient to halt the noxious consequences of hyperglycemia. Such results are in line with most of the clinical data available on the topic [1–3].

Beyond those discussed relatively to the *in vitro* models, our clinical study has several limitations. First, the small sample size impedes the exploration of subgroup analyses. Second, the cohort only included young individuals with T1D and thus observations cannot be extended to people with T2D nor to subjects with longer-duration T1D. Third, the study is cross-sectional, even though CGM recorded the glucose levels before PBMCs collection. A longitudinal design tracking TIR fluctuations and PBMCs changes over time is needed to sustain causality. Fourth, all observational studies are inherently characterized by the risk of residual confounders. To this regard, we did not have information relative to the socioeconomic status of included subjects. Of note, health equity and social determinants of health are major predictors of outcomes, including diabetes complications [29].

Conclusion

In summary, the molecular data presented are consistent with glucose TIR of 70% being sufficient to diminish molecular alterations induced by hyperglycaemia. These findings corroborate existing guidelines recommending a glucose TIR of 70% for people with T1D [1–3].

Abbreviations

TIR	Time in range
HUVEC	Human umbilical vein endothelial cells
SA-βGal	Senescence associated Beta Galactosidase
IL	Interleukin
THP-1	Monocyte cell line
CXCL	Chemokine (C-X-C motif) ligand
PAI	Plasminogen activator inhibitor
TNF	Tumor necrosis factor
MCP	Monocyte chemoattractant protein
NLPR	NLR family pyrin domain containing
ANCOVA	Analysis of covariance
NG	Normal glucose
HG	High glucose
PBMCs	Peripheral blood mononuclear cells

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-025-02983-3.

Supplementary material 1

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Author contributions
R.L.G, A.C., and F.P. conceived the idea and wrote the manuscript. R.L.G., V.P., and F.C. planned and performed most of the experiments, analyzed data, and prepared figures. C.C.B, K.M, B.J.B, K.D., and T.B. provided background expertise, contributed to study design, recruited the cohort, collected samples, and/or critically reviewed the manuscript for its intellectual content.

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Data availability
The datasets analyzed for the study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
Written informed consent was obtained from each participant using the principles of the Helsinki Declaration. The study protocol was approved by the Slovenian Ethical Committee (nr. 0120–6/2023/3).

Consent for publication
Not applicable.

Competing interests
T.B. served on advisory boards of Novo Nordisk, Sanofi, Eli Lilly, Boehringer Ingelheim, Medtronic, Abbott, and Indigo Diabetes. T.B. received honoraria for participating on the speakers' bureau for Eli Lilly, Novo Nordisk, Medtronic, Abbott, Sanofi, Dexcom, Adventis, AstraZeneca, and Roche. T.B.'s institution has received research grant support and travel expenses from Abbott, GluSense, Medtronic, Novo Nordisk, Sanofi, Novartis, Sandoz, and Zealand Pharma. K.D. served on advisory boards for Medtronic and Novo Nordisk and speaker fees from Abbott, Dexcom, Eli Lilly, Novo Nordisk, Medtronic, and Pfizer. A.C. reports on being on Advisory Board, Consultancy, and Lectures: Abbott, Astra Zeneca, Bayer, Berlin Chemie, Boehringer Ingelheim, Hikamr Pharma, Guidotti, Eli Lilly, MSD, Merck, Novo, Roche Diagnostics, Sanofi, Servier, and SUN Pharma. No other potential conflicts of interest relevant to this article were reported.

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