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PAR level mediates the link between ROS and inflammatory response in patients with type 2 diabetes mellitus

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is characterized by disrupted glucose homeostasis and metabolic abnormalities, with oxidative stress and inflammation playing pivotal roles in its pathophysiology. Poly(ADP-ribosyl)ation (PARylation) is a post-translational process involving the addition of ADP-ribose polymers (PAR) to target proteins. While preclinical studies have implicated PARylation in the interplay between oxidative stress and inflammation in T2DM, direct clinical evidence in humans remains limited. This study investigates the relationship between oxidative stress, PARylation, and inflammatory response in T2DM patients.

Methods: This cross-sectional investigation involved 61 T2DM patients and 48 controls. PAR levels were determined in peripheral blood cells (PBMC) by ELISA-based methodologies. Oxidative stress was assessed in plasma and PBMC. In plasma, we monitored reactive oxygen metabolites (d-ROMs) and ferric-reducing antioxidant power. In PBMC, we measured the expression of antioxidant enzymes *SOD1*, *GPX1* and *CAT* by qPCR. Further, we evaluated the expression of inflammatory mediators such as *IL6*, *TNF-α*, *CD68* and *MCP1* by qPCR in PBMC.

Results: T2DM patients exhibited elevated PAR levels in PBMC and increased d-ROMs in plasma. Positive associations were found between PAR levels and d-ROMs, suggesting a link between oxidative stress and altered PAR metabolism. Mediation analysis revealed that d-ROMs mediate the association between HbA1c levels and PAR, indicating oxidative stress as a potential driver of increased PARylation in T2DM. Furthermore, elevated PAR levels were found to be associated with increased expression of pro-inflammatory cytokines *IL6* and *TNF-α* in the PBMC of T2DM patients.

Conclusions: This study highlights that hyperactivation of PARylation is associated with poor glycemic control and the resultant oxidative stress in T2DM. The increase of PAR levels is correlated with the upregulation of key mediators of the inflammatory response. Further research is warranted to validate these findings and explore their clinical implications.

1. Introduction

Type 2 diabetes mellitus (T2DM) poses a significant global health challenge characterized by disrupted glucose homeostasis and associated metabolic abnormalities. Its pathogenesis involves a multifaceted interplay of genetic and environmental factors, culminating in insulin resistance, hyperinsulinemia and impaired pancreatic beta-cell function [1]. Despite extensive research, the precise mechanisms driving T2DM and its complications are, in the main, still undetermined

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Abbreviations

ALT	Alanine Transaminase
AST	Aspartate Transaminase
BMI	body mass index
CAT	Catalase
CD68	Cluster of Differentiation 68 antigen
DBP	diastolic blood pressure
d-ROMs	derivatives of reactive oxygen metabolites
FBG	fasting blood glucose
FRAP	ferric reducing antioxidant power
GGT	Gamma-Glutamyl Transpeptidase
GPX1	Glutathione Peroxidase 1
HbA1c	glycated hemoglobin A1c
IL6	Interleukin 6
MALB	microalbuminuria
MCP1	Monocyte Chemoattractant Protein-1
PAR	poly ADP-ribose
PARylation	poly(ADP-ribosyl)ation
ROS	reactive oxygen species
SBP	systolic blood pressure
SOD1	Superoxide Dismutase 1
T2DM	type 2 diabetes mellitus
TNF-α	Tumor Necrosis Factor Alpha
PBMC	peripheral blood mononuclear cells
PARP	poly(ADP-ribose) polymerase
U CARR	Carratelli units
TPTZ	tripyridyltriazine
FDR	false discovery rate

[2].

Oxidative stress and inflammation are acknowledged as pivotal contributors to the pathophysiology of T2DM and its associated complications. Elevated levels of reactive oxygen species (ROS) and dysregulated inflammatory mediators have been implicated in the development of insulin resistance, beta-cell dysfunction, and vascular complications among diabetic individuals [3–6].

Understanding the intricate relationship between oxidative stress, inflammation, and metabolic dysregulation in T2DM is paramount for identifying new therapeutic targets and for improving patient outcomes.

A central mechanism in the cellular response to oxidative stress is poly(ADP-ribosyl)ation (PARylation), a post-translational process involving the addition of ADP-ribose polymers (PAR) to target proteins by poly(ADP-ribose) polymerases (PARP) [7–9]. PARP-1 accounts for 85 % of the PARP activity in most cellular systems and its activation occurs in response to DNA damage [10]. PARylation plays pivotal roles in DNA repair, multiple cellular homeostasis processes, and gene expression regulation [7–9]. However, hyperactivation of PARP in response to oxidative stress can compromise cellular function and exacerbate disease progression [11,12].

Different studies exploiting cellular and animal models of diabetes have shown elevated levels of PAR to be connected to hyperglycemia and subsequent oxidative stress in various tissues and cells [13–16]. Activation of PARylation has also been confirmed in diabetic patients [17–24], including those with T2DM [18,20–24], as well as in healthy individuals at risk of developing T2DM [23], and is associated with increased oxidative and nitrosative stress [17,19,23].

Excessive PARylation can cause multiple alterations in cellular function, including changes in metabolism and in cell survival, which can lead to long-term dysfunction and tissue damage [25–28]. Of particular interest is the evidence linking increased PARylation to the induction of pro-inflammatory cytokines, the activation of transcription factors NF-κB and AP-1, and the chemotaxis of immune cells [29–31]. In fact, excessive PARP-1 activity has been associated with various inflammatory diseases such as cancer, arthritis, atherosclerosis, and neurodegenerative diseases. Similarly, excessive PARylation may promote a chronic inflammatory state in diabetes, contributing to insulin resistance, vascular damage, and diabetic complications [29–31]. Furthermore, inhibition of PARylation or genetic deletion of PARP-1 can reduce inflammation and alleviate inflammation-associated complications in experimental diabetic animals, as demonstrated in studies on cardiovascular complications [32–34].

Although oxidative stress is a primary trigger for the activation of PARylation and can promote an inflammatory response, the relationship between these molecular aspects has not been comprehensively examined in the clinical setting of T2DM.

The present case-control study investigates the association between oxidative stress, hyperactivation of PARylation, and the consequent alteration of the inflammatory response in individuals with T2DM compared to normoglycemic individuals.

We have previously found in the same cohort that PAR level, indicative of PARP activity, was distinctively enhanced in the peripheral blood cells (PBMC) of T2DM patients, correlating with the severity of glycemic-metabolic imbalance, measured by glycated hemoglobin (HbA1c) [20]. Here, we hypothesize that the observed aberrant PAR metabolism in T2DM patients may stem from dysregulated oxidative stress, potentially leading to the activation of inflammatory pathways.

Firstly, we explored the relationship between PAR levels in PBMC and the antioxidant/oxidant status of T2DM patients. We monitored reactive oxygen metabolites (d-ROMs) and the ferric reducing antioxidant power (FRAP) in plasma. Additionally, we assessed PBMC expression of the antioxidant enzymes Catalase (*CAT*), Superoxide Dismutase 1 (*SOD1*), and Glutathione Peroxidase 1 (*GPX1*), which are crucial for neutralizing ROS within the cells. In particular, we evaluated the potential causal relationship between dysregulated glucose metabolism, elevated PAR levels, and oxidative stress.

Finally, in order to ascertain the existence of a relationship between PARP activation and the inflammatory response, we examined the association between PAR levels and the expression of key inflammatory mediators, such as interleukin-6 (*IL6*), Tumor necrosis factor-alpha (*TNF-α*), Cluster of differentiation 68 (*CD68*) and monocyte chemoattractant protein-1 (*MCP1*) in PBMC from T2DM patients.

Our findings indicate that in T2DM, persistent hyperglycemia is a stressful stimulus capable of increasing oxidative stress, as can be seen both at cellular and plasma level. Cellular responses to stress include increased PARP activity. The increased PAR level emerges as a significant predictor of increased *IL6* and *TNF-α* levels, highlighting its role in regulating inflammatory pathways in T2DM. Therefore PAR may possibly represent an interface between oxidative stress and the activation of inflammatory processes in T2DM.

2. Methods

2.1. Study population

In this case-control study, 109 participants of both sex, aged between 31 and 86 years, were included. The cohort comprised 61 individuals diagnosed with T2DM and 48 healthy controls matched for age and sex. Diabetic patients were recruited from the Diabetes Outpatient Clinic at the Umberto I Hospital, Sapienza University of Rome, Italy, while controls were selected from the Blood Donor Unit of the same hospital.

2.2. Clinical evaluation

Participants underwent comprehensive medical history assessment, clinical examinations, and fasting blood/morning urine collection for routine biochemical and experimental analyses. Anthropometric measurements including weight, height, and waist circumference were recorded. Body Mass Index (BMI) was calculated using weight and height. Systolic and diastolic blood pressure (SBP, DBP) were measured following a 5-min resting period, and the average of the second and third measurements was utilized. Diagnosis of diabetes followed the criteria outlined by the American Diabetes Association in 2020. Among the T2DM patients, 53 out of 61 were receiving treatment with oral glucose-lowering agents such as metformin, incretins, and/or insulin.

Venous blood samples were collected after a 12-h fasting period for analysis of various parameters including blood glucose (FBG), glycosylated hemoglobin (HbA1c), lipid profile (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol calculated using the Friedewald formula), liver enzymes (AST, ALT, GGT), bilirubin (total and conjugated), and creatinine. Morning urine samples were collected for assessment of albuminuria (MALB).

2.3. Isolation of peripheral blood mononuclear cells (PBMC)

Isolation of PBMC was performed as previously described [35]. Briefly, venous blood samples (6 ml) were collected in vacutainer EDTA-containing tubes. Plasma and PBMC were isolated via density gradient centrifugation over the Lymphoprep™ solution (density: 1.077 g/ml) according to the manufacturer's instructions. Isolated plasma and cells were either processed immediately or stored at −80 °C for future use.

2.4. Evaluation of PAR levels in PBMC

To assess the concentration of PAR, nuclear extracts were prepared from freshly collected PBMC samples. PAR quantification was conducted using the HT PARP *in vitro* Pharmacodynamic Assay II kit from Trevigen, following the manufacturer's protocol [36]. Chemiluminescent signals were recorded using the Victor X light plate reader from PerkinElmer Inc. PAR concentrations were determined using the standard curve method, with a standard PAR sample provided in the kit. The standard curve spanned from 10 to 1000 pg/ml, with an r^2 value of 0.993 for the linear curve fit. Each sample was measured in duplicate on experimental plates, and Jurkat cell lysates served as an inter-run calibration sample to ensure consistency across experiments.

2.5. Assessment of oxidative stress in plasma by the d-ROMs test

Plasma levels of d-ROMs were measured using Diacron® commercial colorimetric assay kits (MC013/FREE d-ROMs Test) on the FREE CarpeDiem System analyzer (Diacron®, Grosseto, Italy), according to manufacturer's instructions. The results of the d-ROMs analysis were expressed in Carratelli units (U CARR) where 1 U CARR corresponds to 0.8 mg/l H_2O_2 [37].

In the colorimetric determination, the ROMs (primarily hydroperoxides and ROOH), in presence of iron (that is released from plasma proteins by an acid buffer, the R2 reagent of the kit), are able to generate alkoxyl (R–O°) and peroxyl (R–OO°) radicals, according to the Fenton's reaction. Such radicals, in turn, are able to oxidize an alkyl-substituted aromatic amine (A-NH_2, that is solubilized in a chromogenic mixture, the R1 reagent of the kit) thus transforming them in pink-colored derivatives ($[A\text{-}NH_2^{\circ}]^+$), which are photometrically quantified.

The assay was performed on 20 μl of plasma in 1 ml of specific buffer added of 20 μl of the color-developing chromogen.

2.6. Assessment of antioxidant power of in plasma by the FRAP assay

FRAP assay [38] is a direct measurement of the total reducing power of a sample, representing an indirect index of the system ability to withstand the oxidative damage. It is a colorimetric method based on redox reactions, where antioxidants act as reductant molecules. The reduction of the complex Fe^{3+}- tripyridyltriazine (TPTZ) to the ferrous form Fe^{2+}-TPTZ is performed at low pH. The intense blue color developed during the reaction is monitored by measuring the changes in absorbance at 595 nm by a Sunrise absorbance plate reader (Tecan Italia s.r.l, Segrate, MI). Each sample was analyzed in duplicate. Quantification was obtained by using a Fe^{2+} standard curve and results were expressed in μmol/l.

2.7. Analysis of mRNA expression by RT-qPCR

PBMC pellets, stored at −80 °C, were thawed on ice and subjected to RNA extraction and DNase I digestion using the RNeasy Mini Kit (Qiagen), following the manufacturer's protocol. Assessment of RNA concentration, purity, and integrity was conducted as described previously [39]. Subsequently, reverse transcription was performed utilizing the qPCRBIO cDNA Synthesis Kit (PCR Biosystems) on equivalent amounts of total RNA (1 μg).

Quantitative PCR was carried out to determine specific mRNA levels using the qPCRBIO Master Mix (PCR Biosystems) and the following predesigned primers/probes: Hs_SOD1_2_SG for *SOD1* (QuantiTect, Qiagen); Hs_CAT_1_SG for *CAT* (QuantiTect, Qiagen); Hs_GPX1_1_SG for *GPX1* (QuantiTect, Qiagen); Hs00174128_m1 for *TNF-α* (Thermo Fisher Scientific); Hs02836816_g1 for *CD68* (Thermo Fisher Scientific); Hs00174131_m1 for *IL6* (Thermo Fisher Scientific); Hs00234140_m1for *MCP1* (Thermo Fisher Scientific); and Hs00939627_m1 for *GUSB* (Thermo Fisher Scientific), which served as the reference gene transcript for normalization. An inter-run calibration sample was used in each plate to correct for technical variance between runs and to compare results from different plates. The calibrator consisted of cDNA prepared from HEK293T cells. In each experimental PCR plate, samples were measured in triplicate.

2.8. Statistical analysis

All measurements were conducted at least in triplicate for each participant, and mean values were utilized for subsequent analyses. To assess the distribution of continuous variables, normality was examined using both the Kolmogorov–Smirnov and Shapiro–Wilk tests.

Data are presented as mean ± standard deviation (SD), or percentage, as appropriate. Participants were categorized based on the presence of T2DM, and T2DM patients were further grouped into two consecutive categories based on their levels of PAR, d-ROMs, *IL6*, *MCP1* or *TNF*-α, whether above or below the median value. Statistical comparisons between independent groups were conducted using the Student t-test (normally distributed data) or Mann–Whitney test (non-normally distributed data) for continuous variables, and the χ^2 test for categorical variables. Bivariate correlations were explored using Pearson's r (normally distributed data) or Spearman's ρ (non-normally distributed variables) correlation coefficients.

Multivariable linear regression models were constructed to investigate the relationship between PAR, *IL6*, *MCP1* or *TNF*-α levels (entered as continuous dependent variables) and sex, age, and metabolic parameters (entered as independent variables).

The Baron and Kenny mediation analysis [40] was conducted to investigate whether d-ROMs mediated the relationship between HbA1c levels (predictor variable) and PAR levels (outcome variable) in T2DM patients, with age and sex serving as confounding factors. The significance of a mediation effect was tested by the Sobel method [41,42].

In order to control for false discovery rate (FDR), the Benjamini–Hochberg procedure [43] was applied at a FDR = 0.10.

All statistical analyses were performed using SPSS software (IBM SPSS Statistics Version 23.0).

3. Results

3.1. Study population

For the purposes of this study, we recruited 61 patients with T2DM and 48 normoglycemic individuals who served as a control group (CT). Table 1 presents a comprehensive overview of the key demographic, anthropometric, and clinical characteristics of the study participants. No significant differences in age or sex were observed between the two groups. Individuals with T2DM exhibited markedly higher body mass index (BMI), waist circumference, and systolic blood pressure (SBP). Regarding plasma metabolic parameters, T2DM patients had significantly higher levels of fasting blood glucose (FBG), glycated hemoglobin (HbA1c), and triglycerides. Total cholesterol, LDL cholesterol, and HDL cholesterol were significantly lower than those reported in the control group in line with the prevalence of statins treatment in T2DM patients (Statins treated individuals were 6 % of the CT and 33 % of the T2DM group, $p = 0.005$ after Pearson chi-square test).

Additionally, T2DM patients demonstrated significantly higher gamma-glutamyl transferase (GGT) levels compared to controls.

3.2. Oxidative stress markers and their association with PAR levels in T2DM patients

T2DM patients displayed higher levels of PAR in PBMC (CT = 63.96 ± 38.04 pg/ml, T2DM = 108.38 ± 70.57 pg/ml, $p = 0.001$, $q = 0.010$), along with higher plasma levels of d-ROMs (CT = 293.60 ± 16.93 U CARR, T2DM = 369.06 ± 36.16 U CARR, $p < 0.001$, $q = 0.010$), compared to control individuals. Moreover, T2DM patients exhibited increased expression of *CAT* (CT = 1.88 ± 0.66 AU, T2DM = 2.53 ± 1.15 AU, $p = 0.009$, $q = 0.064$), and *SOD1* (CT = 0.19 ± 0.10 AU, T2DM = 0.24 ± 0.11 AU, $p = 0.013$, $q = 0.085$) mRNA in PBMC (Table 1).

When stratifying the T2DM cohort according to PAR levels (above and below the median PAR value of 86.87 pg/ml), we observed significantly higher d-ROMs (low PAR = 357.76 ± 29.90 U CARR, high PAR = 400.78 ± 33.29 U CARR, $p = 0.007$, $q = 0.050$) and lower FRAP (low PAR = 587.57 ± 65.23 µmol/l, high PAR = 490.16 ± 83.36 µmol/l, $p = 0.021$, $q > 0.10$) in T2DM individuals with greater PAR levels (Table S1, Fig. 1). Bivariate correlation analysis confirmed the positive correlation between PAR and d-ROMs ($r = 0.64$, $p < 0.001$; $q = 0.010$) (Table S2).

In multivariate analysis, increased d-ROMs emerged as the sole significant predictor of PAR ($\beta = 0.77$, $p = 0.017$) in T2DM patients, independently of age, sex, BMI, FBG, and HbA1c levels ($F(6,13) = 5.388$, $p = 0.005$, $r^2 = 0.713$) (Table 2).

3.3. Clinical and oxidative stress variables in relation to d-ROMs level in T2DM patients

When stratifying the T2DM cohort according to d-ROMs levels (above and below the median d-ROMs value of 368 U CARR), elevated d-ROMs were associated with increased FBG (low d-ROMs = 123.50 ± 29.64 mg/dl, high d-ROMs = 155.87 ± 42.95 mg/dl, $p = 0.008$, $q = 0.058$) and HbA1c levels (low d-ROMs = 6.85 ± 0.95 %, high d-ROMs = 7.82 ± 1.41 %, $p = 0.015$, $q = 0.093$), as well as reduced *CAT* mRNA levels in PBMC (low d-ROMs = 2.71 ± 1.06, high d-ROMs = 1.97 ± 0.63, $p = 0.047$, $q > 0.10$) (Table S3). Bivariate correlation analysis confirmed the association of d-ROMs with FBG, ($\rho = 0.46$, $p = 0.007$, $q = 0.054$), HbA1c ($\rho = 0.45$, $p = 0.008$, $q = 0.058$) and *CAT* mRNA ($r = -0.42$, $p = 0.031$, $q > 0.10$), while indicated that higher d-ROMs were also linked to decreased FRAP ($r = -0.43$, $p = 0.020$, $q > 0.10$) and *SOD1* mRNA expression ($r = -0.39$, $p = 0.047$, $q > 0.10$) (Table S4).

3.4. Mediation analysis for the relationship between PAR and HbA1c via d-ROMs in T2DM patients

The existence of a positive correlation between PAR and HbA1c [20], together with evidence suggesting a positive association between both variables and d-ROMs, led to the hypothesis that d-ROMs could mediate the relationship between elevated HbA1c levels and PAR in T2DM patients. This hypothesis was tested using Baron and Kenny's three-step mediation analysis method [40], with age and sex included as confounding variables (Fig. 2). In step 1 of the mediation model, increased

Table 1
Characteristics of the study population.

	CT	T2DM	*P*
N	48	61	
Age (years)	60 ± 8	63 ± 9	0.065
Male % (n)	48 (23)	62 (38)	0.174
Disease duration (years)	-	8.51 ± 7.47	
BMI (kg/m^2)	26.12 ± 3.77	29.85 ± 5.14	**< 0.001***
Waist circ. (cm)	84 ± 8.1	106.8 ± 11.69	**< 0.001***
DBP (mm Hg)	79.09 ± 7.70	81.33 ± 11.08	0.311
SBP (mm Hg)	124.76 ± 10.39	139.35 ± 18.65	**< 0.001***
FBG (mg/dl)	88.69 ± 10.98	143.13 ± 41.04	**< 0.001***
HbA1c (%)	5.25 ± 0.35	7.58 ± 1.39	**< 0.001***
Total cholesterol (mg/dl)	213.18 ± 35.27	181.86 ± 39.56	**< 0.001***
LDL cholesterol (mg/dl)	124.65 ± 30.82	91.29 ± 45.61	**< 0.001***
HDL cholesterol (mg/dl)	64.41 ± 14.78	46.41 ± 11.41	**< 0.001***
Triglycerides (mg/dl)	100.11 ± 33.53	177.56 ± 83.39	**< 0.001***
ALT (U/l)	19.93 ± 6.40	22.67 ± 13.51	0.184
AST (U/l)	21.37 ± 5.00	19.42 ± 8.49	0.154
GGT (U/l)	16.94 ± 5.79	28.51 ± 21.40	**0.004***
Total bilirubin (mg/dl)	0.57 ± 0.06	0.58 ± 0.27	0.929
Direct bilirubin (mg/dl)	0.19 ± 0.01	0.22 ± 0.01	0.624
Creatinine (mg/dl)	0.89 ± 0.17	0.90 ± 0.32	0.744
MALB (mg/l)	–	24.95 ± 29.61	
PAR (pg/ml)	63.96 ± 38.04	108.38 ± 70.57	**0.001***
d-ROMs (U CARR)	293.60 ± 16.93	369.06 ± 36.16	**< 0.001***
FRAP (µmol/l)	565.40 ± 103.86	563.32 ± 83.93	0.389
CAT mRNA (AU)	1.88 ± 0.66	2.53 ± 1.15	**0.009***
SOD1 mRNA (AU)	0.19 ± 0.10	0.24 ± 0.11	**0.013***
GPX1 mRNA (AU)	85.90 ± 50.44	82.29 ± 45.62	0.369
CD68 mRNA (AU)	35.76 ± 10.33	57.23 ± 16.79	**0.001***
IL6 mRNA (AU)	1.25 ± 0.69	1.09 ± 0.84	0.674
MCP1 mRNA (AU)	1.23 ± 0.70	1.13 ± 0.49	0.669
TNF-α mRNA (AU)	1.29 ± 0.82	1.20 ± 0.48	0.756

p value: Student t-test or Mann–Whitney *U* test (continuous variables) and chi-square test (prevalence, for categorical variables). Bold text indicates significant *p* values (≤0.05). The asterisk (*) indicates significant difference after the Benjamini-Hochberg correction procedure at a false discovery rate (FDR) of 0.1. Values are mean ± SD for continuous variables; percentage (number) for categorical variables.

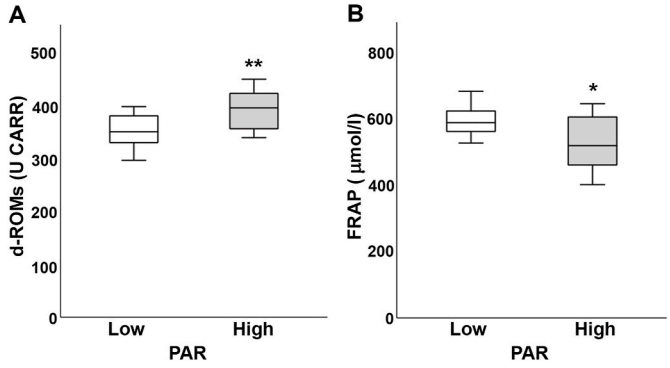

Fig. 1. Levels of d-ROMs and FRAP in low and high PAR T2DM patients. Box-whisker plot showing the level of d-ROMs (A) and FRAP (B) in plasma of T2DM patients. Boxes show the median, the 25th and the 75th percentiles. Whiskers show the minimum and the maximum data point. The asterisk (*) indicates significant difference after the Student t-test. *$p < 0.05$; **$p < 0.01$.

Table 2
Multivariate regression analysis. PAR level is the dependent variable.

	Unst. Coeff.	Std. Coeff.	
	B	Beta	*P*
(constant)	−329.03		0.093
Age	0.80	0.19	0.378
Sex	6.61	0.06	0.795
BMI	−0.21	−0.02	0.941
FBG	−0.66	−0.53	0.051
HbA1c	−1.80	−0.04	0.851
d-ROMs	1.17	0.77	**0.017**
FRAP	0.08	0.12	0.700

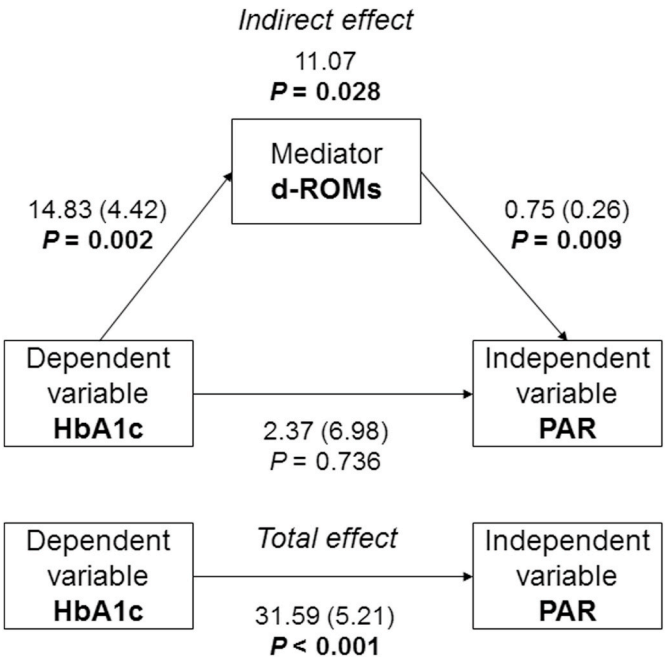

Fig. 2. Mediation analysis model showing that d-ROMs mediate the effect of HbA1c on PAR level in T2DM patients. Path diagram for the indirect effect of HbA1c levels (independent variable) on PAR levels (dependent variable) through the mediator variable d-ROMs (upper panel) and for the total effect of the independent variable on the dependent variable (lower panel). Data are standardized regression coefficients, standard error and *p* value.

HbA1c significantly predicted higher PAR level (β = 31.59, $p < 0.001$, total effect). Step 2 revealed that increased HbA1c also significantly predicted higher d-ROMs (β = 14.83, $p = 0.002$). Finally, in step 3 HbA1c ceased to be a significant predictor of PAR level when d-ROMs were included as a control variable (β = 2.37, $p = 0.736$), while d-ROMs emerged as a significant positive predictor of PAR level (β = 0.75, $p = 0.009$). Consequently, as the effect of HbA1c on PAR vanished completely, d-ROMs were identified as fully mediating the effect of HbA1c on PAR. This mediation was statistically significant in the Sobel test [41,42] (β = 11.07, $p < 0.028$, indirect effect).

3.5. Inflammatory mediators in relation to PAR levels in T2DM patients

The comparison of mRNA expression levels of key inflammatory mediators in PBMC of T2DM patients, stratified based on PAR levels, demonstrated an association between elevated PAR level and heightened expression of *IL6* (low PAR = 0.57 ± 0.25 AU, high PAR = 1.77 ± 0.82, $p < 0.001$, $q = 0.010$) (Table S5 and Fig. 3). Bivariate correlation analysis confirmed the positive association between PAR and *IL6* mRNA level (ρ = 0.82, $p < 0.001$, $q = 0.010$), further highlighting a positive association of PAR with *MCP1* (ρ = 0.38, $p = 0.021$, $q > 0.10$) and *TNF-α* (r = 0.43, $p = 0.011$, $q = 0.074$) mRNA levels (Table S6).

Several additional variables, beyond PAR, have been found to be associated with *IL6*, *MCP1*, and *TNF-α* levels in T2DM patients after stratification and correlation analysis. In T2DM patients stratified based on *IL6* level (above and below the median *IL6* mRNA value), elevated *IL6* expression was associated with higher HbA1c (low *IL6* = 6.97 ± 0.77 %, high *IL6* = 8.48 ± 1.71 %, $p = 0.002$, $q = 0.012$) (Table S7). Bivariate correlation confirmed higher HbA1c in association with higher *IL6* (ρ = 0.46, $p = 0.005$, $q = 0.044$) (Table S8).

In T2DM patients stratified based on *MCP1* level (above and below the median *MCP1* mRNA value), we observed a positive association of *MCP1* expression with FBG (low *MCP1* = 121.78 ± 32.93 mg/dl, high *MCP1* = 160.47 ± 33.30 mg/dl, $p = 0.001$, $q = 0.010$), HbA1c (low *MCP1* = 6.74 ± 0.91 %, high *MCP1* = 8.53 ± 1.43 %, $p < 0.001$, $q = 0.010$), GGT (low *MCP1* = 23.67 ± 11.32 U/l high *MCP1* = 48.33 ± 30.82 U/l, $p = 0.046$, $q > 0.10$) and MALB (low *MCP1* = 5.67 ± 5.58 mg/l, high *MCP1* = 31.01 ± 22.19 mg/l, $p < 0.001$, $q = 0.010$). In contrast, we found a negative association with mRNA of *SOD1* (low *MCP1* = 0.29 ± 0.12 AU, high *MCP1* = 0.20 ± 0.09 AU, $p = 0.029$, $q > 0.10$.) and *CAT* (low *MCP1* = 3.08 ± 1.46 AU, high *MCP1* = 1.93 ± 0.83 AU, $p = 0.015$, $q = 0.093$). Bivariate correlation analysis confirmed these associations (Table S8). In contrast, *TNF-α* mRNA level did not show any further association beyond the linear correlation with PAR mentioned above (Table S8).

Multivariate linear regression models were then built to assess the predictive performance of PAR with respect to the variables associated with *IL6*, *MCP1*, and *TNF-α* levels (Tables S7 and S8) in T2DM patients. Regression models, adjusted for age, sex, BMI, and FBG, showed that PAR significantly predicted *IL6* levels (β = 0.789, $p < 0.001$), independently of HbA1c levels ($F(6,21) = 6.632$, $p < 0.001$, $r^2 = 0.655$)

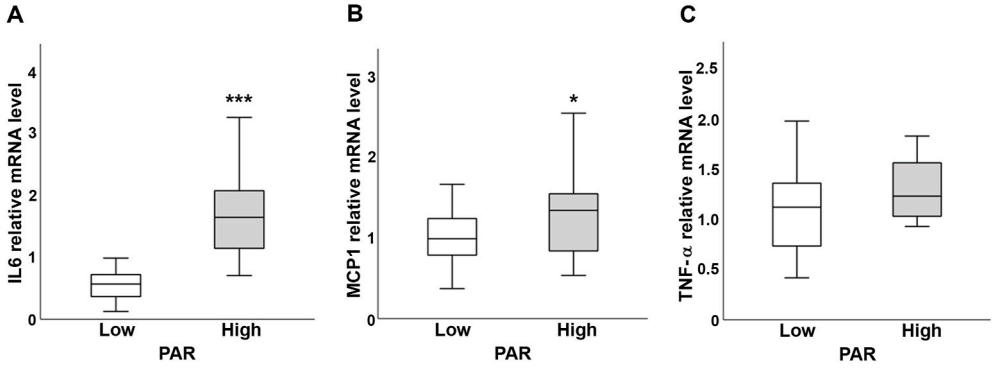

Fig. 3. Transcript level of inflammation mediators in low and high PAR T2DM patients. Box-whisker plot showing the mRNA level of *IL6* (A), *MCP1* (B) and *TNF-α* (C) in PBMC of T2DM patients. Boxes show the median, the 25th and the 75th percentiles. Whiskers show the minimum and the maximum data point. The asterisk (*) indicates significant difference after the Student t-test. *$p < 0.05$; ***$p < 0.001$.

Table 3
Multivariate regression analysis. *IL6*, *TNF-α* and *MCP1* mRNA levels are the dependent variables.

	Unst. Coeff.	Std. Coeff.	
	B	Beta	*P*
(constant)	0.807		0.549
Age	−0.007	−0.084	0.584
Sex	−0.111	−0.059	0.685
BMI	−0.011	−0.068	0.625
FBG	0.001	0.048	0.756
HbA1c	0.005	0.009	0.966
PAR	0.009	0.789	**< 0.001**
Dependent variable: *IL6*			
(constant)	0.583		0.573
Age	0.006	0.123	0.579
Sex	−0.119	−0.109	0.596
BMI	0.003	0.029	0.89
FBG	−0.002	−0.133	0.518
PAR	0.004	0.514	**0.018**
Dependent variable: *TNF-α*			
(constant)	−0.648		0.381
Age	−0.006	−0.134	0.384
Sex	0.063	0.062	0.668
BMI	0.007	0.075	0.593
FBG	0.004	0.301	0.067
HbA1c	0.195	0.624	**0.006**
PAR	0.061	−0.035	0.851
Dependent variable: *MCP1*			

(Table 3), and TNF-α level (β = 0.514, $p = 0.018$) in T2DM patients ($F(5,20) = 1.553$, $p = 0.041$, $r^2 = 0.603$) (Table 3).

Variables associated with *MCP1* were pre-selected by running a stepwise multivariate regression, with HbA1c identified as the primary predictor beyond PAR levels. Subsequently, the predictive performance of PAR and HbA1c for *MCP1* in T2DM patients was tested using multivariate regression analysis, adjusted for age, sex, BMI, and FBG ($F(6,23) = 6.148$, $p < 0.001$, $r^2 = 0.616$). HbA1c level was found to predict *MCP1* levels independently of PAR levels (β = 0.624, $p = 0.006$) (Table 3).

4. Discussion

The main finding of this study is that the inefficient glycemic control in T2DM patients increases oxidative stress both at cellular and plasma levels and is associated with the activation of PARylation and upregulation of *IL6* and *TNF-α* expression. This put forward a role of PARylation in interfacing oxidative stress with the inflammatory pathways in T2DM.

Preclinical studies, conducted both *in vitro* and on animal models, have demonstrated that in the context of diabetes, PARylation acts as a key mediator between oxidative stress, resulting from prolonged hyperglycemia, and the activation of a cascade of mediators that facilitate the inflammatory process [16,23,28,44–49]. However, direct clinical evidence on this correlation in human T2DM was limited up to this study.

Consistently with data describing the activation of PARylation as a result of persistent hyperglycemia [49–51], we previously demonstrated, in the same case cohort as the present study, that high plasma HbA1c levels (>7.5 %) can predict higher PAR levels in the PBMC of T2DM patients [20]. Other studies reported that activation of PARP enzymes and accumulation of PAR were associated with DNA damage caused by oxidative stress, due to high glucose [16,49,51].

Here, we examine the association between increased PAR levels and oxidative stress in T2DM patients compared to controls and the influence of elevated PAR in the inflammatory response.

T2DM patients exhibit higher d-ROMs levels, molecules containing hydroperoxide groups, reflecting an excess of ROS in plasma. Furthermore, an increase in *CAT, SOD1*, and *GPX1* expression, encoding key antioxidant enzymes was detected in PBMC. This could represent a compensatory mechanism of blood cells in response to elevated oxidative stress. These data are in line with previous studies that have outlined an association between oxidative stress and T2DM [6,52].

However, the elevation of PAR levels in PBMC is associated with plasma markers of oxidative stress rather than cellular ones. In fact, as indicated by the positive association between PAR and d-ROMs and the negative association of PAR with FRAP, higher PAR levels appear to be correlated with an imbalance in the ratio of oxidants to antioxidants, favoring oxidants in the plasma of T2DM patients.

Contrary to expectations, PAR levels do not correlate with *CAT* and *SOD1* expression, despite these enzymes being elevated in T2DM patients compared to controls. This discrepancy may be due to the complexity of the oxidative stress response and the distinct roles of PARylation and antioxidant enzymes. Cells use various mechanisms to counteract excess ROS, depending on the damage and context. PARylation mainly aids DNA repair, while SOD1 and CAT detoxify ROS. Both processes also regulate signaling pathways and other cellular functions [53,54]. Variations in signaling pathways in T2DM and individual patient differences (e.g., genetics, metabolic control, comorbidities) may further explain the lack of association between PAR levels and *CAT* and *SOD1* expression.

Significantly, d-ROMs were found to be the main predictor of higher PAR levels in T2DM patients, independently of clinical variables associated with T2DM, including HbA1c. This suggests a strong link between PAR metabolism and systemic oxidative stress inT2DM.

Higher d-ROMs levels in T2DM patients were associated with increased FBG and HbA1c. In addition, d-ROMs were negatively associated with plasma FRAP and expression of *CAT* and *SOD1* in PBMC. Taken together, these data suggest that higher d-ROMs in T2DM patients are linked to persistent hyperglycemia and compromised antioxidant defense both in plasma and in cells.

The positive association of both d-ROMs and PAR with HbA1c, and the observation that d-ROMs act as a confounding factor in the association between PAR and HbA1c, prompted us to evaluate a potential cause-effect relationship among these three variables. Mediation analysis confirmed this hypothesis, indicating that the positive association between HbA1c and PAR is mediated by d-ROMs. These results are consistent with the proposed mechanism for the activation of PARP enzymes in diabetes, in which they directly respond to ROS- induced DNA strand breaks where there is persistent hyperglycemia [16,49,51].

PARP enzymes act as a “cellular rheostat” that, as a consequence of stress signals, can promote diverse cellular responses [8,11]. Inflammation is a tissue response to stress caused by hyperglycemia, and PARylation is an important mediator in the inflammatory response [29,53,54]. It has been proposed that long-term, low-level chronic inflammation associated with the onset and/or worsening of several diseases, including diabetes, is largely dependent on PARylation, as demonstrated in various experimental models based on PARP enzyme inhibition or genetic deletion [54–56]. In line with these observations, studies on T2DM patients indicate that molecules that can counteract PARP enzymes activation show efficacy in attenuating cytokine release by blood cells [57] and in improving skin vascular function [58].

In effect, PARP-1 enzyme is reported to act as a coactivator of transcription factors, such as NF-κB, AP1, and AP2, promoting the development of inflammatory processes through the upregulation of various inflammatory mediators, including *TNF-α*, *MCP1*, and several interleukins like *IL6* [59–64]. Accordingly, we observed that higher PAR levels are associated with increased expression of *IL6*, *TNF-α* and *MCP1* in PBMC from T2DM patients. Significantly, PAR emerged as an independent predictor of *TNF-α* and *IL6* expression, suggesting that PARylation plays a role in activating the pro-inflammatory response in the clinical context of diabetes. However, we cannot rule-out that the increase in PAR level we observed is partly a consequence of inflammation. In fact, it has been proposed that inflammation itself can cause

PARylation activation through an increase in ROS production. This can lead to a vicious cycle in which inflammation further stimulates PARylation, which in turn amplifies inflammation [27,54,65].

Despite the significant findings and implications of this study, some limitations should be acknowledged. Firstly, although PBMC offer insights into immune cell-mediated processes, our data may not fully reflect the dynamics of PARylation and inflammation in target tissues relevant to T2DM pathophysiology, such as pancreatic β-cells and adipose tissue.

Secondly, due to the cross-sectional design of the study, future analyses, including longitudinal studies, are necessary to clarify the temporal relationships and causal pathways underlying the association between oxidative stress, dysregulated PARylation, and inflammatory response in subjects with T2DM. The observed associations are based on correlations, thus we cannot rule out the possibility of reverse causality, as inflammation may also cause oxidative stress and increased PARylation. Furthermore, the increase in PAR, cytokines, and oxidant levels may simply be a coincidental consequence of a more severe disease. Although we controlled for confounding variables, residual confounding remains possible, considering factors that may influence oxidative stress like medication, diet, and physical activity. Finally, the study mainly used mRNA levels of antioxidant enzymes and inflammatory mediators, potentially overlooking protein activities.

5. Conclusions

This study sheds light on the intricate interplay between oxidative stress, dysregulated PARylation, and the inflammatory response in individuals with T2DM. Our findings underscore the pivotal role of oxidative stress in driving aberrant PAR production, which may in turn contribute to the activation of inflammatory pathways in T2DM. Our study shows 1) a link between elevated PAR levels in PBMC of T2DM patients and increased oxidative stress markers in plasma, highlighting the association between hyperglycemia, oxidative stress, and increased PARylation; 2) an association between PAR levels and the expression of key inflammatory mediators such as *IL6*, and *TNF*-α in PBMC of T2DM patients, underscoring a possible role of PARylation in modulating the inflammatory response.

These results may pave the way for future research efforts focusing on the development of new therapeutic interventions targeting PARP activity in T2DM management.

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Ethics approval and consent to participate

The study was reviewed and approved by the Ethics Committee of Policlinico Umberto I, Sapienza University of Rome (ID#3550, 2015, 26th Feb.) and was conducted in conformance with the Helsinki Declaration. A written informed consent was obtained from the subjects before participating in the study.

CRediT authorship contribution statement

Michele Zampieri: Writing – review & editing, Writing – original draft, Visualization, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Katsyarina Karpach:** Writing – review & editing, Visualization, Investigation. **Gerardo Salerno:** Writing – review & editing, Investigation. **Anna Raguzzini:** Writing – review & editing, Investigation. **Ilaria Barchetta:** Writing – review & editing, Formal analysis, Data curation. **Flavia Agata Cimini:** Writing – review & editing, Data curation. **Sara Dule:** Writing – review & editing, Data curation. **Giovanna De Matteis:** Writing – review & editing, Investigation. **Giuseppe Zardo:** Writing – review & editing, Investigation. **Marina Borro:** Writing – review & editing. **Ilaria Peluso:** Writing – review & editing, Supervision. **Maria Gisella Cavallo:** Writing – review & editing, Supervision, Conceptualization. **Anna Reale:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2024.103243.

References

[1] S.E. Kahn, M.E. Cooper, S. Del Prato, Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future, Lancet (London, England) 383 (2014) 1068–1083, https://doi.org/10.1016/S0140-6736(13)62154-6.
[2] American Diabetes Association, 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2020, Diabetes Care 43 (2020) S14–S31, https://doi.org/10.2337/dc20-S002.
[3] J.L. Evans, I.D. Goldfine, B.A. Maddux, G.M. Grodsky, Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes 52 (2003) 1–8, https://doi.org/10.2337/diabetes.52.1.1.
[4] J.C. Pickup, M.A. Crook, Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 41 (1998) 1241–1248, https://doi.org/10.1007/s001250051058.
[5] J.L. Rains, S.K. Jain, Oxidative stress, insulin signaling, and diabetes, Free Radic. Biol. Med. 50 (2011) 567–575, https://doi.org/10.1016/j.freeradbiomed.2010.12.006.
[6] M.Y. Donath, S.E. Shoelson, Type 2 diabetes as an inflammatory disease, Nat. Rev. Immunol. 11 (2011) 98–107, https://doi.org/10.1038/nri2925.
[7] M.O. Hottiger, P.O. Hassa, B. Lüscher, H. Schüler, F. Koch-Nolte, Toward a unified nomenclature for mammalian ADP-ribosyltransferases, Trends Biochem. Sci. 35 (2010) 208–219, https://doi.org/10.1016/j.tibs.2009.12.003.
[8] B.A. Gibson, W.L. Kraus, New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs, Nat. Rev. Mol. Cell Biol. 13 (2012) 411–424, https://doi.org/10.1038/nrm3376.
[9] A. Bürkle, L. Virág, Poly(ADP-ribose): PARadigms and PARadoxes, Mol. Aspect. Med. 34 (2013) 1046–1065, https://doi.org/10.1016/j.mam.2012.12.010.
[10] V. Schreiber, F. Dantzer, J.-C. Ame, G. de Murcia, Poly(ADP-ribose): novel functions for an old molecule, Nat. Rev. Mol. Cell Biol. 7 (2006) 517–528, https://doi.org/10.1038/nrm1963.
[11] P. Bai, Biology of poly(ADP-ribose) polymerases: the factotums of cell maintenance, Mol. Cell 58 (2015) 947–958, https://doi.org/10.1016/j.molcel.2015.01.034.
[12] L. Virág, C. Szabó, The therapeutic potential of poly(ADP-ribose) polymerase inhibitors, Pharmacol. Rev. 54 (2002) 375–429, https://doi.org/10.1124/pr.54.3.375.
[13] V. Burkart, Z.Q. Wang, J. Radons, B. Heller, Z. Herceg, L. Stingl, E.F. Wagner, H. Kolb, Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozocin, Nat. Med. 5 (1999) 314–319, https://doi.org/10.1038/6535.
[14] P. Bai, C. Cantó, The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease, Cell Metabol. 16 (2012) 290–295, https://doi.org/10.1016/j.cmet.2012.06.016.
[15] P. Pacher, C. Szabo, Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease, Am. J. Pathol. 173 (2008) 2–13, https://doi.org/10.2353/ajpath.2008.080019.
[16] F. Garcia Soriano, L. Virág, P. Jagtap, E. Szabó, J.G. Mabley, L. Liaudet, A. Marton, D.G. Hoyt, K.G. Murthy, A.L. Salzman, G.J. Southan, C. Szabó, Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation, Nat. Med. 7 (2001) 108–113, https://doi.org/10.1038/83241.

[17] E.M. Horváth, R. Magenheim, E. Kugler, G. Vácz, A. Szigethy, F. Lévárdi, M. Kollai, C. Szabo, Z. Lacza, Nitrative stress and poly(ADP-ribose) polymerase activation in healthy and gestational diabetic pregnancies, Diabetologia 52 (2009) 1935–1943, https://doi.org/10.1007/s00125-009-1435-3.
[18] A. Giorgi, I. Tempera, G. Napoletani, D. Drovandi, C. Potestà, S. Martire, E. Mandosi, T. Filardi, M. Eugenia Schininà, S. Morano, M. D'Erme, B. Maras, Poly (ADP-ribosylated) proteins in mononuclear cells from patients with type 2 diabetes identified by proteomic studies, Acta Diabetol. 54 (2017) 833–842, https://doi.org/10.1007/s00592-017-1013-y.
[19] E.M. Horváth, R. Magenheim, N.J. Béres, R. Benkő, T. Pék, Á.G. Tabák, C. Szabo, Oxidative-nitrative stress and poly (ADP-Ribose) polymerase activation 3 Years after pregnancy, Oxid. Med. Cell. Longev. 2018 (2018) 1743253, https://doi.org/10.1155/2018/1743253.
[20] M. Zampieri, M.G. Bacalini, I. Barchetta, S. Scalea, F.A. Cimini, L. Bertoccini, S. Tagliatesta, G. De Matteis, G. Zardo, M.G. Cavallo, A. Reale, Increased PARylation impacts the DNA methylation process in type 2 diabetes mellitus, Clin. Epigenetics 13 (2021) 114, https://doi.org/10.1186/s13148-021-01099-1.
[21] A. Adaikalakoteswari, M. Rema, V. Mohan, M. Balasubramanyam, Oxidative DNA damage and augmentation of poly(ADP-ribose) polymerase/nuclear factor-kappa B signaling in patients with type 2 diabetes and microangiopathy, Int. J. Biochem. Cell Biol. 39 (2007) 1673–1684, https://doi.org/10.1016/j.biocel.2007.04.013.
[22] Q.-A.A. Altaf, A. Ali, M.K. Piya, N.T. Raymond, A.A. Tahrani, The relationship between obstructive sleep apnea and intra-epidermal nerve fiber density, PARP activation and foot ulceration in patients with type 2 diabetes, J. Diabet. Complicat. 30 (2016) 1315–1320, https://doi.org/10.1016/j.jdiacomp.2016.05.025.
[23] C. Szabo, A. Zanchi, K. Komjati, P. Pacher, A.S. Krolewski, W.C. Quist, F. W. LoGerfo, E.S. Horton, A. Veves, Poly(ADP-Ribose) polymerase is activated in subjects at risk of developing type 2 diabetes and is associated with impaired vascular reactivity, Circulation 106 (2002) 2680–2686. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12438293.
[24] N.-H. Cui, J.-M. Yang, X. Liu, X.-B. Wang, Poly(ADP-Ribose) polymerase activity and coronary artery disease in type 2 diabetes mellitus: an observational and bidirectional mendelian randomization study, Arterioscler. Thromb. Vasc. Biol. 40 (2020) 2516–2526, https://doi.org/10.1161/ATVBAHA.120.314712.
[25] S.A. Andrabi, G.K.E. Umanah, C. Chang, D.A. Stevens, S.S. Karuppagounder, J.-P. Gagné, G.G. Poirier, V.L. Dawson, T.M. Dawson, Poly(ADP-ribose) polymerase-dependent energy depletion occurs through inhibition of glycolysis, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 10209–10214, https://doi.org/10.1073/pnas.1405158111.
[26] M. Brownlee, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813–820, https://doi.org/10.1038/414813a.
[27] F. Giacco, M. Brownlee, Oxidative stress and diabetic complications, Circ. Res. 107 (2010) 1058–1070, https://doi.org/10.1161/CIRCRESAHA.110.223545.
[28] X. Du, T. Matsumura, D. Edelstein, L. Rossetti, Z. Zsengellér, C. Szabó, M. Brownlee, Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells, J. Clin. Invest. 112 (2003) 1049–1057, https://doi.org/10.1172/JCI18127.
[29] S. Pazzaglia, C. Pioli, Multifaceted role of PARP-1 in DNA repair and inflammation: pathological and therapeutic implications in cancer and non-cancer diseases, Cells 9 (2019), https://doi.org/10.3390/cells9010041.
[30] E.K. Lee, P.A. Konstantinopoulos, PARP inhibition and immune modulation: scientific rationale and perspectives for the treatment of gynecologic cancers, Ther. Adv. Med. Oncol. 12 (2020) 1758835920944116, https://doi.org/10.1177/1758835920944116.
[31] X. Ba, N.J. Garg, Signaling mechanism of poly(ADP-ribose) polymerase-1 (PARP-1) in inflammatory diseases, Am. J. Pathol. 178 (2011) 946–955, https://doi.org/10.1016/j.ajpath.2010.12.004.
[32] M. Kassan, S.-K. Choi, M. Galán, A. Bishop, K. Umezawa, M. Trebak, S. Belmadani, K. Matrougui, Enhanced NF-κB activity impairs vascular function through PARP-1-, SP-1-, and COX-2-dependent mechanisms in type 2 diabetes, Diabetes 62 (2013) 2078–2087, https://doi.org/10.2337/db12-1374.
[33] E.M. Zakaria, H.M. El-Bassossy, N.N. El-Maraghy, A.F. Ahmed, A.A. Ali, PARP-1 inhibition alleviates diabetic cardiac complications in experimental animals, Eur. J. Pharmacol. 791 (2016) 444–454, https://doi.org/10.1016/j.ejphar.2016.09.008.
[34] P. Li, Y. Wang, X. Liu, B. Liu, Z.-Y. Wang, F. Xie, W. Qiao, E.-S. Liang, Q.-H. Lu, M.-X. Zhang, Loss of PARP-1 attenuates diabetic arteriosclerotic calcification via Stat1/Runx2 axis, Cell Death Dis. 11 (2020) 22, https://doi.org/10.1038/s41419-019-2215-8.
[35] M.G. Bacalini, A. Reale, M. Malavolta, F. Ciccarone, M. Moreno-Villanueva, M.E. T. Dollé, E. Jansen, T. Grune, E.S. Gonos, C. Schön, J. Bernhardt, B. Grubeck-Loebenstein, E. Sikora, O. Toussaint, F. Debacq-Chainiaux, M. Capri, A. Hervonen, M. Hurme, P.E. Slagboom, N. Breusing, V. Aversano, S. Tagliatesta, C. Franceschi, M.A. Blasco, A. Bürkle, P. Caiafa, M. Zampieri, Ageing affects subtelomeric DNA methylation in blood cells from a large European population enrolled in the MARK-AGE study, GeroScience 43 (2021) 1283–1302, https://doi.org/10.1007/s11357-021-00347-9.
[36] G. De Matteis, A. Reale, F. Grandoni, M.L. Meyer-Ficca, M.C. Scatà, R.G. Meyer, L. Buttazzoni, B. Moioli, Assessment of Poly(ADP-ribose) Polymerase1 (PARP1) expression and activity in cells purified from blood and milk of dairy cattle, Vet. Immunol. Immunopathol. 202 (2018) 102–108, https://doi.org/10.1016/j.vetimm.2018.06.013.
[37] N. Brkljača Bottegaro, J. Gotić, J. Šuran, D. Brozić, K. Klobučar, K. Bojanić, Z. Vrbanac, Effect of prolonged submaximal exercise on serum oxidative stress biomarkers (d-ROMs, MDA, BAP) and oxidative stress index in endurance horses, BMC Vet. Res. 14 (2018) 216, https://doi.org/10.1186/s12917-018-1540-y.
[38] I.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1996) 70–76, https://doi.org/10.1006/abio.1996.0292.
[39] M.G. Bacalini, S. Tavolaro, N. Peragine, M. Marinelli, S. Santangelo, I. Del Giudice, F.R. Mauro, V. Di Maio, M.R. Ricciardi, P. Caiafa, S. Chiaretti, R. Foà, A. Guarini, A. Reale, A subset of chronic lymphocytic leukemia patients display reduced levels of PARP1 expression coupled with a defective irradiation-induced apoptosis, Exp. Hematol. 40 (2012) 197–206.e1, https://doi.org/10.1016/j.exphem.2011.11.005.
[40] R.M. Baron, D.A. Kenny, The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations, J. Pers. Soc. Psychol. 51 (1986) 1173–1182, https://doi.org/10.1037//0022-3514.51.6.1173.
[41] M.E. Sobel, Asymptotic confidence intervals for indirect effects in structural equation models, Socio. Methodol. 13 (1982) 290, https://doi.org/10.2307/270723.
[42] M.E. Sobel, Some new results on indirect effects and their standard errors in covariance structure models, Socio. Methodol. 16 (1986) 159, https://doi.org/10.2307/270922.
[43] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. R. Stat. Soc. Ser. B Stat. Methodol. 57 (1995) 289–300, https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.
[44] N.A. Berger, V.C. Besson, A.H. Boulares, A. Bürkle, A. Chiarugi, R.S. Clark, N. J. Curtin, S. Cuzzocrea, T.M. Dawson, V.L. Dawson, G. Haskó, L. Liaudet, F. Moroni, P. Pacher, P. Radermacher, A.L. Salzman, S.H. Snyder, F.G. Soriano, R. P. Strosznajder, B. Sümegi, R.A. Swanson, C. Szabo, Opportunities for the repurposing of PARP inhibitors for the therapy of non-oncological diseases, Br. J. Pharmacol. 175 (2018) 192–222, https://doi.org/10.1111/bph.13748.
[45] I.G. Obrosova, F. Li, O.I. Abatan, M.A. Forsell, K. Komjáti, P. Pacher, C. Szabó, M. J. Stevens, Role of poly(ADP-ribose) polymerase activation in diabetic neuropathy, Diabetes 53 (2004) 711–720, https://doi.org/10.2337/diabetes.53.3.711.
[46] L. Zheng, C. Szabó, T.S. Kern, Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-kappaB, Diabetes 53 (2004) 2960–2967, https://doi.org/10.2337/diabetes.53.11.2960.
[47] C. Szabó, A. Biser, R. Benko, E. Böttinger, K. Suszták, Poly(ADP-ribose) polymerase inhibitors ameliorate nephropathy of type 2 diabetic Leprdb/db mice, Diabetes 55 (2006) 3004–3012, https://doi.org/10.2337/db06-0147.
[48] E.M. Horváth, R. Benko, L. Kiss, M. Murányi, T. Pék, K. Fekete, T. Bárány, A. Somlai, A. Csordás, C. Szabo, Rapid "glycaemic swings" induce nitrosative stress, activate poly(ADP-ribose) polymerase and impair endothelial function in a rat model of diabetes mellitus, Diabetologia 52 (2009) 952–961, https://doi.org/10.1007/s00125-009-1304-0.
[49] W.-D. Qin, G.-L. Liu, J. Wang, H. Wang, J.-N. Zhang, F. Zhang, Y. Ma, X.-Y. Ji, C. Li, M.-X. Zhang, Poly(ADP-ribose) polymerase 1 inhibition protects cardiomyocytes from inflammation and apoptosis in diabetic cardiomyopathy, Oncotarget 7 (2016) 35618–35631, https://doi.org/10.18632/oncotarget.8343.
[50] S. Li, J. Deng, D. Sun, S. Chen, X. Yao, N. Wang, J. Zhang, Q. Gu, S. Zhang, J. Wang, S. Zhu, H. Zhu, H. Li, X. Xu, F. Wei, FBXW7 alleviates hyperglycemia-induced endothelial oxidative stress injury via ROS and PARP inhibition, Redox Biol. 58 (2022) 102530, https://doi.org/10.1016/j.redox.2022.102530.
[51] X. Du, T. Matsumura, D. Edelstein, L. Rossetti, Z. Zsengeller, C. Szabo, M. Brownlee, Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells, J. Clin. Invest. 112 (2003) 1049–1057. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14523042.
[52] A. Caturano, M. D'Angelo, A. Mormone, V. Russo, M.P. Mollica, T. Salvatore, R. Galiero, L. Rinaldi, E. Vetrano, R. Marfella, M. Monda, A. Giordano, F.C. Sasso, Oxidative stress in type 2 diabetes: impacts from pathogenesis to lifestyle modifications, Curr. Issues Mol. Biol. 45 (2023) 6651–6666, https://doi.org/10.3390/cimb45080420.
[53] Y. Ke, C. Wang, J. Zhang, X. Zhong, R. Wang, X. Zeng, X. Ba, The role of PARPs in inflammation-and metabolic-related diseases: molecular mechanisms and beyond, Cells 8 (2019), https://doi.org/10.3390/cells8091047.
[54] P. Bai, L. Virag, Role of poly(ADP-ribose) polymerases in the regulation of inflammatory processes, FEBS Lett. 586 (2012) 3771–3777. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23022557.
[55] E.M. Zakaria, N.N. El-Maraghy, A.F. Ahmed, A.A. Ali, H.M. El-Bassossy, PARP inhibition ameliorates nephropathy in an animal model of type 2 diabetes: focus on oxidative stress, inflammation, and fibrosis, Naunyn-Schmiedeberg's Arch. Pharmacol. 390 (2017) 621–631, https://doi.org/10.1007/s00210-017-1360-9.
[56] D. V Santinelli-Pestana, E. Aikawa, S.A. Singh, M. Aikawa, PARPs and ADP-ribosylation in chronic inflammation: a focus on macrophages, Pathogens 12 (2023), https://doi.org/10.3390/pathogens12070964.
[57] A.R. Weseler, L. Geraets, H.J.J. Moonen, R.J.F. Manders, L.J.C. van Loon, H.-J. Pennings, E.F.M. Wouters, A. Bast, G.J. Hageman, Poly (ADP-ribose) polymerase-1-inhibiting flavonoids attenuate cytokine release in blood from male patients with chronic obstructive pulmonary disease or type 2 diabetes, J. Nutr. 139 (2009) 952–957, https://doi.org/10.3945/jn.108.102756.
[58] G. Shrikhande, L. Khaodhiar, S. Scali, C. Lima, M. Hubbard, K. Dudley, O. Ganda, C. Ferran, A. Veves, Valsartan improves resting skin blood flow in type 2 diabetic patients and reduces poly(adenosine diphosphate-ribose) polymerase activation, J. Vasc. Surg. 43 (2006) 760–770, https://doi.org/10.1016/j.jvs.2005.12.059. ; discussion 770-1.

[59] P.O. Hassa, M.O. Hottiger, The functional role of poly(ADP-ribose)polymerase 1 as novel coactivator of NF-kappaB in inflammatory disorders, Cell. Mol. Life Sci. 59 (2002) 1534–1553, https://doi.org/10.1007/s00018-002-8527-2.

[60] P.O. Hassa, S.S. Haenni, C. Buerki, N.I. Meier, W.S. Lane, H. Owen, M. Gersbach, R. Imhof, M.O. Hottiger, Acetylation of poly(ADP-ribose) polymerase-1 by p300/CREB-binding protein regulates coactivation of NF-kappaB-dependent transcription, J. Biol. Chem. 280 (2005) 40450–40464, https://doi.org/10.1074/jbc.M507553200.

[61] S. Krug, A.A. Ordonez, M. Klunk, B.G. Kang, S.K. Jain, T.M. Dawson, V.L. Dawson, W.R. Bishai, Host regulator PARP1 contributes to sex differences and immune responses in a mouse model of tuberculosis, bioRxiv 4 (21) (2021) 440820, https://doi.org/10.1101/2021.04.21.440820, 2021.

[62] D. Kovács, V.B. Vántus, E. Vámos, N. Kálmán, R. Schicho, F. Gallyas, B. Radnai, Olaparib: a clinically applied PARP inhibitor protects from experimental crohn's disease and maintains barrier integrity by improving bioenergetics through rescuing glycolysis in colonic epithelial cells, Oxid. Med. Cell. Longev. 2021 (2021) 7308897, https://doi.org/10.1155/2021/7308897.

[63] R.R. Gerner, V. Klepsch, S. Macheiner, K. Arnhard, T.E. Adolph, C. Grander, V. Wieser, A. Pfister, P. Moser, N. Hermann-Kleiter, G. Baier, H. Oberacher, H. Tilg, A.R. Moschen, NAD metabolism fuels human and mouse intestinal inflammation, Gut 67 (2018) 1813–1823, https://doi.org/10.1136/gutjnl-2017-314241.

[64] F.A. Kunze, M. Bauer, J. Komuczki, M. Lanzinger, K. Gunasekera, A.-K. Hopp, M. Lehmann, B. Becher, A. Müller, M.O. Hottiger, ARTD1 in myeloid cells controls the IL-12/18-IFN-γ Axis in a model of sterile sepsis, chronic bacterial infection, and cancer, J. Immunol. 202 (2019) 1406–1416, https://doi.org/10.4049/jimmunol.1801107.

[65] X. Luo, W.L. Kraus, On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1, Genes Dev. 26 (2012) 417–432, https://doi.org/10.1101/gad.183509.111.