VIA MEDICA
PRACA ORYGINALNA
ISSN 2084–4441

Iga A. Turek[1], Lucyna A. Wozniak[1], Katarzyna Cypryk[2, 3], Iwona Nadel[3, 4], Marzena Wojcik[1]

[1]Department of Structural Biology, Faculty of Biomedical Sciences and Postgraduate Education, Medical University of Lodz, Poland
[2]Diabetology and Metabolic Diseases Department, Medical University of Lodz, Poland
[3]Endocrinology and Metabolic Diseases Department, Polish Mother's Memorial Hospital Research Institute in Lodz, Poland
[4]Diabetological Medical Center "OmniMed" in Lodz, Poland

Evaluation of leukocyte *SIRT1* expression in women with gestational diabetes mellitus (GDM) in the third trimester of pregnancy

Ocena leukocytarnej ekspresji *SIRT1* u kobiet ze zdiagnozowaną cukrzycą ciążową (GDM) w trzecim trymestrze ciąży

ABSTRACT

Background. Sirtuin 1 (SIRT1), a NAD^+-dependent deacetylase, has been implicated as a key regulator of glucose/lipid metabolism, insulin secretion as well as adiponectin production and inflammation in metabolic disorders, including type 2 diabetes mellitus (T2DM). However, its role in gestational diabetes mellitus (GDM) remains widely unknown. Since GDM is associated with inflammation, the aim of this study was to determine whether leukocyte *SIRT1* mRNA expression is altered in GDM women in the third trimester of pregnancy, and whether this change is correlated with clinical characteristics of patients.

Methods. Leukocytes were isolated from the blood of GDM (n = 135) and normal glucose tolerant (NGT; n = 52) pregnant women. After extracting RNA from leukocytes, a quantitative real-time polymerase chain reaction (qRT-PCR) approach was performed to assess *SIRT1* gene expression in these cells. Univariate regression analyses were applied to investigate correlations between *SIRT1* expression and clinic parameters of patients.

Results. Leukocyte *SIRT1* mRNA was increased 1.7-fold in the GDM *vs.* NGT subjects (p = 0.001) and it positively correlated with 2 h glucose concentration during oral glucose tolerance test (OGTT) in the whole study group and negatively correlated with pregnancy age in the GDM and NGT groups. The positive association was also observed between *SIRT1* mRNA and plasma high density lipoprotein (HDL) cholesterol level in the NGT subjects.

Conclusions. GDM is accompanied by leukocyte *SIRT1* mRNA overexpression associated with hyperglycemia. Additionally, there is a close and beneficial relationship between enhanced leukocyte *SIRT1* expression and increased plasma HDL-cholesterol level during normal pregnancy. (Diabet. Klin. 2014; 3, 1: 3–11)

Key words: gestational diabetes mellitus (GDM), sirtuin 1 (SIRT1), oxidative stress, inflammation, type 2 diabetes mellitus (T2DM)

STRESZCZENIE

Wstęp. Sirtuina 1 (SIRT1) jest NAD^+-zależną deacetylazą, która odgrywa istotną rolę w regulacji metabolizmu węglowodanów i lipidów, sekrecji insuliny, produkcji

Adres do korespondencji:
dr n. chem. Marzena Wojcik
Department of Structural Biology, Faculty of Biomedical Sciences and Postgraduate Education
Medical University of Lodz, 7/9 Zeligowskiego St., 90–752 Lodz
Tel: +48 (42) 639 32 38
Fax: +48 (42) 639 32 21
e-mail: marzena.wojcik@umed.lodz.pl
Diabetologia Kliniczna 2014, tom 3, nr 1, 3–11

Nadesłano: 30.12.2013 Przyjęto do druku: 04.01.2014

adiponektyny oraz stanu zapalnego w chorobach metabolicznych, w tym cukrzycy typu 2 (T2DM). Do tej pory brak jest informacji o związku leukocytarnej SIRT1 z cukrzycą ciążową (GDM). Zważywszy związek SIRT1 ze stanem zapalnym, celem badania było określenie zmian w poziomie ekspresji *SIRT1* mRNA w leukocytach kobiet z GDM w trzecim trymestrze ciąży i skorelowanie ich z klinicznymi parametrami pacjentek.

Materiały i metody. Leukocyty wyizolowano z krwi pobranej od kobiet ciężarnych z GDM (n = 135) oraz kobiet ciężarnych z prawidłową gospodarką węglowodanową (NGT; n = 52). Po ekstrakcji RNA z leukocytów poziom ekspresji *SIRT1* mRNA w tych komórkach określono metodą ilościowego qRT-PCR. Korelacje między ekspresją *SIRT1* a klinicznymi parametrami pacjentek analizowano z wykorzystaniem regresji jednokrotnych.

Wyniki. Ekspresja *SIRT1* była 1,7-krotnie wyższa w leukocytach kobiet z GDM niż w grupie kobiet z NGT ($p = 0{,}001$) i dodatnio korelowała ze stężeniem glukozy w 2. godzinie testu doustnego obciążenia glukozą (OGTT) w całej badanej populacji pacjentek oraz ujemnie korelowała z wiekiem ciąży kobiet zarówno w grupie GDM, jak i NGT ($p < 0{,}05$). Stwierdzono również istotną statystycznie dodatnią korelację między ekspresją *SIRT1* a stężeniem cholesterolu frakcji HDL w grupie NGT ($p < 0{,}05$).

Wnioski. GDM towarzyszy podwyższona ekspresja *SIRT1* w leukocytach, która jest związana z hiperglikemią. Ponadto zaobserwowano istnienie bliskiego i korzystnego związku między podwyższonymi poziomami ekspresji *SIRT1* a cholesterolem frakcji HDL u kobiet z prawidłowym przebiegiem ciąży. (Diabet. Klin. 2014; 3, 1: 3–11)

Słowa kluczowe: cukrzyca ciążowa (GDM), sirtuina 1 (SIRT1), stres oksydacyjny, stan zapalny, cukrzyca typu 2 (T2DM)

Introduction

Sirtuin 1 (SIRT1), a nicotine adenine dinucleotide (NAD^+)-dependent protein deacetylase, is the most extensively studied of mammalian silent information regulator 2 proteins (sirtuins) in metabolic disorders, including type 2 diabetes mellitus (T2DM) [1]. It is well known that SIRT1 is broadly distributed in mammalian tissues and its transcriptional level is regulated in response to calorie restriction or fasting. SIRT1 has been implicated in the regulation of glucose and lipid metabolism as well as insulin secretion and inflammation by deacetylating various transcription factors and transcriptional co-regulatory proteins in major metabolic tissues. It has been demonstrated that SIRT1 inhibits fat storage and increases lipolysis in adipose tissue *via* repression of PPARγ (peroxisome proliferator-activated receptor gamma), an essential regulator of adipogenesis and fat storage that affects the expression of many adipocyte-specific genes [2]. SIRT1 also induces gluconeogenesis and inhibits glycolysis in the liver by deacetylating PGC-1α (peroxisome proliferator--activated receptor gamma coactivator 1 alpha) under low-nutrient conditions [3] and, additionally, enhances glucose-stimulated insulin secretion in pancreatic β-cells through down-regulation of UCP2 (uncoupling protein 2) [4]. In skeletal muscle, under low glucose level, SIRT1 deacetylates PGC-1α causing the activation of genes involved in mitochondrial fatty acid oxidation, electron transport, and oxidative phosphorylation; thereby it can act as a sensor of nutrient adaptation through inducing fatty acid oxidation in response to low glucose concentration [5]. Furthermore, SIRT1 inhibits inflammation in adipocytes, macrophages, and hepatocytes through the suppression of transcriptional activity of NF-κB (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1) resulting from deacetylation of RelA/p65 subunit of NF-κB [6–9].

GDM is defined as hyperglycemia with onset or first recognition during pregnancy that affects from 3% to 17% of all pregnancies depending on race and ethnicity [10]. GDM is associated with numerous and serious complications for both mother (i.e. preeclampsia, preterm delivery, cesarean section, pregnancy-induced hypertension as well as elevated risk of developing T2DM and cardiovascular disease after pregnancy) and fetus (i.e. prematurity, macrosomia, hypoglycemia, jaundice, respiratory distress syndrome, polycythemia, and hypocalcemia) [11].

Insulin resistance and pancreatic β-cell dysfunction are considered as two major determinants of GDM development [12]. Although the pathophysiology of GDM remains still unclear, several factors associated with this metabolic disorder have been identified. Among them, increased oxidative stress and inflammation as well as hyperleptinemia and hypoadiponectinemia are now most recognized [13–19]. Thus, GDM is a highly complex process involving multiple factors, and their identification along with elucidation of mechanisms underlying GDM is a serious challenge.

Despite the fact that a number of *in vitro* and *in vivo* studies have shown the link of SIRT1 with T2DM [20, 21], no study exists so far on the role of leukocyte SIRT1 in GDM, thus, no data are available on its potential association with anthropometric and metabolic parameters of diabetic pregnant women. Therefore, we sought to determine whether *SIRT1* gene expression is

altered in leukocytes of the GDM women in the third trimester of pregnancy and whether this change is correlated with clinical characteristics of patients. In the current study, we employed leukocytes obtained from the GDM patients as the experimental cellular model, since these cells are known to play a key role in pathogenetic processes linked to diabetes such as inflammation. In addition, the use of leukocytes allowed circumventing the invasive and non-ethical procedures involved in taking metabolic tissue samples from pregnant women.

Material and methods

Subjects

This study compared GDM women in the third trimester of pregnancy (24–33 weeks) with pregnant women with similar characteristics but without GDM. One hundred thirty five women with GDM and 52 pregnant women with NGT (control group) were recruited to the study from the Diabetological Medical Center "OmniMed" in Lodz, Poland.

All women underwent a 75 g oral glucose tolerance test (OGTT). GDM was diagnosed according to the criteria established by the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study (at least one value is met or exceeded of the following thresholds during the 75 g OGTT: fasting plasma glucose 92 mg/dL, 1 h plasma glucose 180 mg/dL, or 2 h plasma glucose 153 mg/dL) [10]. The inclusion criteria were the following:

- diagnosis of GDM according to the criteria set by the HAPO study;
- Caucasian ethnic background;
- age range between 18 and 44 years;
- no family history of diabetes within first-degree relatives;
- no GDM in a previous pregnancy;
- absence of pre-pregnancy diabetes;
- absence of concomitant systemic disease (chronic or acute or infectious);
- no treatment with insulin or oral hypoglycemic agents.

The diet and exercise were not controlled before overnight fast. All clinical investigations were conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethical Committee of the Medical University of Lodz (No. RNN-186-11-KF from 20.09.2011). Informed consent was obtained from all participating subjects.

Clinical measurements

The information on maternal age and pre-pregnancy weight were collected from medical records. The weight and height of patients during pregnancy were measured using standard methods, and body weight gain as well as pre-pregnancy body mass index (BMI), expressed as weight before pregnancy divided by squared height (kg/m^2), were calculated. Gestational age was established based on the last menstrual period. Systolic (SBP) and diastolic (DBP) blood pressure were also measured using an electronic monitor, after 10 minutes of rest in a sitting position.

Blood samples were collected from the patients between 7.30 a.m. and 9.30 a.m. after a 12-hour overnight fast. Serum triglycerides (TGs), and HDL- and LDL-cholesterol levels were determined by enzymatic colorimetric methods with the triglyceride GPO-PAP and the Total Cholesterol CHOD-PAP kits (Roche Diagnostics, Mannheim, Germany). The oral glucose tolerance test (OGTT) was carried out with the standardized 75 g glucose solution. The glycated hemoglobin (HbA_{1C}) was measured by a latex enhanced turbidimetric immunoassay using specific monoclonal antibodies. The biochemical assays were carried out on COBAS INTEGRA analyzer (Roche, SA). The concentration of C-reactive protein (CRP) was determined by turbidimetric assay with the use of the cassette COBAS INTEGRA C-Reactive Protein (Latex) according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). Plasma insulin was quantified using Elecsys insulin assay (Roche Diagnostics GmbH, Mannheim, Germany). Insulin resistance and β-cell function were estimated by homeostasis model assessment HOMA-IR and HOMA-B, respectively [22].

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mg/dL)}] / 405$$

$$\text{HOMA-B} = [360 \times \text{fasting insulin } (\mu\text{U/mL})] / [\text{fasting glucose (mg/dL)} - 63]$$

To assess insulin sensitivity, the quantitative insulin sensitivity check index (QUICKI-IS) was calculated as follows: QUICKI-IS = 1/[log (I0) + log (G0)], where I0 is the fasting plasma insulin concentration (μU/mL) and G0 is the fasting blood glucose concentration (mg/dL) [23].

RNA Extraction and Quantitative Real-Time PCR

Leukocytes were isolated from heparinized blood of the patients according to previously described procedure [24]. Total RNA was extracted from leukocytes using commercially available acid-phenol reagent (Tri-Reagent® Solution, Ambion, USA) according to the manufacturer's instructions. RNA concentration was quantified using a LAMBDA 25 UV spectrophotometer (PerkinElmer, UK). RNA quality and integrity were determined through the $UV_{260/280}$ ratio. Four micro-

gram of RNA was converted to cDNA in the presence of $(dT)_{18}$ primer and RevertAid™ H Minus M-MuLV reverse transcriptase, according to the manufacturer's protocol (Fermentas, Lithuania). The cDNA was diluted 10-fold, and 2 μL of cDNA was used to perform qRT-PCR using Maxima™ SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific, USA) and specific primers for *SIRT1* (forward: 5'-GCTCGCCTTGCTGTAGACTT-3' and reverse: 5'-TGTGACAGAGAGATGGCTGG-3') and for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as a housekeeping gene (forward: 5'-GGTGGTCTCCTCTGACTTCAACA-3'and reverse: 5'-GTTGCTGTAGCCAAATTCGTTGT-3'). Amplification was carried out on 7500 Real Time PCR System (Applied Biosystems, USA) with initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 60 s. All samples were run in duplicate. Specificity of the product was assessed from the melting curve analysis.

Following baseline correction, the fluorescence threshold level was set during the exponential phase of PCR amplification to generate the threshold cycle (C_t) value for each amplification curve. The ΔC_t value between *SIRT1* target and endogenous control *GAPDH* was calculated as: $\Delta Ct = Ct^{(SIRT1)} - Ct^{(GAPDH)}$. The expression changes between the GDM and NGT groups were expressed as $\Delta\Delta Ct$ and calculated in the following manner: $\Delta\Delta Ct = \Delta Ct^{(GDM)} - \Delta Ct^{(NGT)}$. The fold change value between the two groups was determined as $2^{-\Delta\Delta Ct}$ [25].

Statistical analysis

The distribution of analyzed data was checked by the Shapiro-Wilk test. Variables with distribution different than normal were transformed logarithmically to examine whether they have a log-normal distribution. Differences between variables were calculated using the Student's *t* test (in the case of normally and log-normally distributed data) or the non-parametric Mann-Whitney U (Wilcoxon) test (in the case of non-Gaussian distribution data). The correlations between *SIRT1* mRNA expression and each clinical parameter value were determined by the nonparametric test of Spearman's rank correlation coefficient. The *P* value < 0.05 was considered as statistically significant. Data are expressed as mean value ± standard deviation (SD). Statistical analysis was performed using a commercially available statistical software package (Statistica version 10.0, StatSoft, Poland, license no AXAP301E504323AR-B).

Results

Subjects' characteristics

The clinical characteristics of 135 GDM and 52 NGT pregnant women are presented in Table 1. As expected, maternal plasma glucose concentrations at 0 h, 1 h and 2 h during 75 g OGTT as well as HbA_{1C} levels were significantly higher in the GDM group compared with the NGT controls ($p < 0.05$). Additionally, the GDM patients had markedly lower plasma HDL-cholesterol levels than the NGT controls ($p < 0.05$). No significant differences existed in both maternal and gestational age, blood pressure (SBP and DBP), and the parameters of maternal adiposity (i.e. pre- and pregnancy BMI, body weight gain), lipid metabolism (i.e. TGs, LDL-cholesterol and total cholesterol), inflammation (CRP), insulin resistance/sensitivity (i.e. insulin level, HOMA-IR/QUICKI-IS), and insulin secretion (HOMA-B) between the two groups ($p > 0.05$).

Leukocyte *SIRT1* mRNA expression and correlations

The alterations in leukocyte *SIRT1* gene expression in the GDM ($n = 135$) vs. the NGT ($n = 52$) subjects were determined by qRT-PCR. The *SIRT1* mRNA level in these cells was significantly higher in the GDM group compared with the control group (0.016 ± 0.010 vs. 0.011 ± 0.008, $p = 0.001$ as assessed by the non-parametric Mann-Whitney U test) with a 1.7-fold up-regulation (Fig. 1).

To better describe the relationship between SIRT1 and GDM, we evaluated the association of *SIRT1* gene expression with individual clinical parameters of patients given in Table 1. Univariate correlation analyses with the use of the Spearman rank test revealed that *SIRT1* mRNA positively associated with 2 h OGTT glucose in the whole study group ($r = 0.16$, $p = 0.035$) (Tab. 2, Fig. 2A) and negatively correlated with pregnancy age both in the GDM and NGT pregnant women ($r = -0.22$, $p = 0.015$ and $r = -0.32$, $p = 0.027$, respectively) as well as the entire study group ($r = -0.20$, $p = 0.008$) (Tab. 2, Fig. 2B–2D). There was also the significant positive association of *SIRT1* mRNA with plasma HDL-cholesterol level in the NGT group ($r = 0.30$, $p = 0.039$) (Tab. 2, Fig. 2E). There were no correlations between leukocyte *SIRT1* gene expression and other measured variables (Tab. 2).

Discussion

To the best of our knowledge, it is the first study concerning the evaluation of leukocyte *SIRT1* mRNA expression in the GDM group vs. the NGT controls and its correlations with clinical characteristics of the patients, in an attempt to investigate the potential significance of SIRT1 in GDM.

The group of 135 pregnant women with GDM and 52 NGT pregnant women at the third trimester of gestation was included in the study. The GDM women had significantly higher post-load glucose and HbA_{1C} levels and lower HDL-cholesterol concentration.

Table 1. Clinical characteristics of the NGT and GDM groups

Parameter	NGT group (n = 52)	GDM group (n = 135)	p
Age (year)	30.98 ± 4.58	30.96 ± 4.77	0.920[a]
Pregnancy age (week)	28.57 ± 2.45	28.28 ± 2.48	0.438[b]
Pre-pregnancy BMI [kg/m^2]	25.38 ± 5.02	27.00 ± 6.04	0.099[b]
Body weight gain [kg]	8.93 ± 4.41	8.50 ± 4.66	0.283[b]
TGs [mg/dL]	201.97 ± 67.93	206.50 ± 60.58	0.460[a]
Total cholesterol [mg/dL]	266.25 ± 43.11	259.81 ± 43.40	0.345[a]
HDL-cholesterol [mg/dL]	82.04 ± 17.34	74.77 ± 15.14	0.008[a]*
LDL-cholesterol [mg/dL]	146.53 ± 36.08	147.16 ± 41.32	0.888[a]
HbA_{1C} [%]	5.28 ± 0.24	5.40 ± 0.28	0.008[a]*
Glucose 0 h [mg/dL]	81.16 ± 6.29	86.97 ± 13.55	0.004[b]*
Glucose 1 h [mg/dL]	147.82 ± 31.76	179.15 ± 25.29	< 0.001[b]**
Glucose 2 h [mg/dL]	135.33 ± 21.76	162.03 ± 23.62	< 0.001[b]**
SBP [mm Hg]	122.02 ± 12.53	125.11 ± 14.68	0.210[a]
DBP [mm Hg]	72.96 ± 9.61	74.36 ± 10.77	0.465[a]
CRP [mg/dL]	4.59 ± 5.44	4.57 ± 4.09	0.557[b]
Insulin [μIU/mL]	11.82 ± 17.41	10.98 ± 7.28	0.867[a]
HOMA-IR	2.21 ± 3.43	2.20 ± 1.66	0.059[b]
HOMA-B	176 ± 97	181 ± 122	0.739[a]
QUICKI-IS	0.36 ± 0.04	0.35 ± 0.06	0.059[b]
SIRT1	0.011 ± 0.008	0.016 ± 0.010	0.001[b]**

BMI — body mass index; CRP — C reactive protein; DBP — diastolic blood pressure; HbA_{1C} — glycated hemoglobin; HDL — high-density lipoprotein; HOMA-B — homeostasis model assessment of β-cell function; HOMA-IR — homeostasis model assessment of insulin resistance; HR — heart rate; LDL — low-density lipoprotein; SBP — systolic blood pressure; QUICKI-IS — quantitative insulin sensitivity check index; TGs — triglycerides. Data represent the mean ± SD

*p < 0.01; **p < 0.001 as compared to control as assessed by the Student t test ([a]) or the Mann-Whitney U test ([b]) for normally or log-normally and non-normally distributed data, respectively

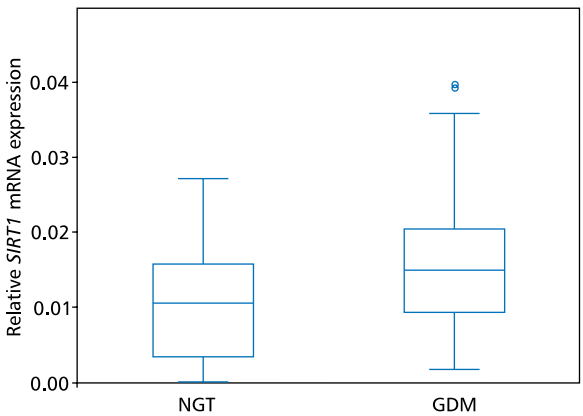

Figure 1. Boxplots of *SIRT1* mRNA expression in the NGT (n = 52) and GDM (n = 135) groups. Middle line: median; box: interquartile range; whisker: range (excluding outliers); p = 0.001 compared with the NGT group by the Mann-Whitney U test

In the current study, we found a 1.7-fold increase in leukocyte *SIRT1* mRNA in the GDM women compared with the NGT pregnant control in the third trimester of pregnancy, suggesting the existence of a linkage of GDM to a change in SIRT1 at its gene expression. These results are in contrast to previous studies showing reduced *SIRT1* expression in various cells obtained from diabetic patients as well as in several obese and T2DM animal experimental models. For instance, Song et al. [20] reported leukocyte *SIRT1* down-regulation in T2DM patients aged 45–75 years from the control non-diabetic group, who differed in several clinical parameters, among others BMI. SIRT1 mRNA and protein levels were also significantly decreased in epididymal fat from insulin resistance obese and *db*/*db* diabetic mice [26]. Moreover, Sun et al. [27] showed markedly decreased SIRT1 protein level in the gastrocnemius muscle of mice with impaired glucose tolerance compared to NGT mice. Additionally, the authors provided evidence that SIRT1 was directly involved in the modulation of insulin sensitivity as a negative regulator of protein tyrosine phosphatase 1 B (PTP1B) transcription at the chromatin level. Recent studies in this field have supported the concept that reduced SIRT1 gene and protein expression is linked to enhanced insulin resistance of subjects [28]. Taking into account the aforementioned studies, it may be hypothesized that the elevated leukocyte *SIRT1* expression in the diabetic pregnant women observed in our study is not related to the clinical variab-

Table 2. Univariate correlations of *SIRT1* expression with clinical characteristic of patients

Parameter	*SIRT1* whole group		*SIRT1* NGT group		*SIRT1* GDM group	
	r	p	r	p	r	p
Age (year)	0.05	0.513	0.18	0.226	0.00	0.968
Pregnancy age (week)	–0.26	< 0.001*	–0.32	0.027*	–0.22	0.015*
Pre-pregnancy BMI [kg/m^2]	0.02	0.806	–0.10	0.476	0.02	0.809
Body weight gain [kg]	–0.11	0.140	–0.11	0.437	–0.10	0.235
TGs [mg/dL]	–0.01	0.947	–0.22	0.133	0.07	0.402
Total cholesterol [mg/dL]	0.09	0.252	0.06	0.664	0.13	0.136
HDL-cholesterol [mg/dL]	0.01	0.926	0.30	0.039*	–0.03	0.716
LDL-cholesterol [mg/dL]	0.10	0.163	–0.04	0.789	0.16	0.069
HbA_{1C} [%]	–0.09	0.219	–0.08	0.572	–0.17	0.057
Glucose 0 h [mg/dL]	–0.01	0.946	–0.01	0.927	–0.06	0.492
Glucose 1 h [mg/dL]	0.17	0.067	0.10	0.595	–0.05	0.671
Glucose 2 h [mg/dL]	0.16	0.035*	0.02	0.902	0.03	0.722
SBP [mm Hg]	0.11	0.130	0.01	0.926	0.11	0.192
DBP [mm Hg]	0.04	0.605	–0.17	0.257	0.07	0.407
CRP [mg/dL]	–0.02	0.832	–0.16	0.284	0.02	0.838
Insulin [μIU/mL]	0.08	0.279	–0.07	0.656	0.11	0.236
HOMA–IR	0.05	0.537	–0.10	0.528	0.05	0.543
HOMA–B	0.14	0.073	0.06	0.725	0.18	0.053
QUICKI–IS	–0.05	0.537	0.10	0.528	–0.05	0.543

r- and p-values are given. Abbreviations are indicated in Table 1. *Significant correlation as assessed by the Spearman's correlation method

les such as age, obesity, and insulin resistance of the patients since the GDM and NGT groups were similar in term of maternal age as well as the parameters of maternal adiposity (i.e. pre- and pregnancy BMI, body weight gain) and insulin resistance/sensitivity (i.e. insulin level, HOMA-IR/QUICKI-IS).

The diet calorie restriction and physical activity are proved to be major stimulus for a change in SIRT1 expression in the insulin-dependent tissues [29]. However, 12 h fasting of the subjects during the current study appears to be too short to detect increased leukocyte *SIRT1* in the GDM women [29]. Additionally, since the NGT and GDM pregnant women enrolled in this study did not received any recommendations regarding exercise and diet before the overnight fast, thus the two groups of patients did not differ between each other in this respect, the impact of these factors on leukocyte *SIRT1* overexpression in the GDM group cannot be considered.

Although alterations in leukocyte SIRT1 expression have not been yet addressed in GDM, they might be related to hyperglycemic conditions, particularly, since the significant positive correlation between leukocyte *SIRT1* mRNA and 2 h OGTT glucose was found in the present study. Growing evidence links GDM to hyperglycemia-induced oxidative stress, which appears when there is an imbalance between generation of reactive oxygen species (ROS) and its clearance by the antioxidant defense systems [13, 30]. Several mechanisms underlying this phenomenon have been proposed, including the polyol pathway, formation of advanced glycation end products (AGEs), the hexosamine pathway, activation of protein kinase C (PKC), and enhanced ROS production in the mitochondria [13]. In the last few years, a number of *in vitro* and *in vivo* studies have been published on the protective role of SIRT1 overexpression against oxidative stress. For example, Hasegawa et al. [31] demonstrated SIRT1 up-regulation with the enhancement of catalase expression in renal tubular cells under ROS-stimulated conditions. Additionally, low to moderate (2.5- to 7.5-fold) cardiac-specific SIRT1 overexpression in transgenic mice protected the heart against oxidative stress through an increase of catalase expression by the forkhead box O proteins (FoxO)-dependent mechanism [32]. Interestingly, a greater increase in SIRT1 expression (12.5-fold) induced cardiomyopathy [32]. SIRT1 protective function against hyperglycemia-induced oxidative stress was also reported in pancreatic beta cells [33]. Taking into account these findings, it is reasonable to assume that leukocyte *SIRT1* overexpression under hyperglycemia in the GDM women might be a regulatory adaptation of

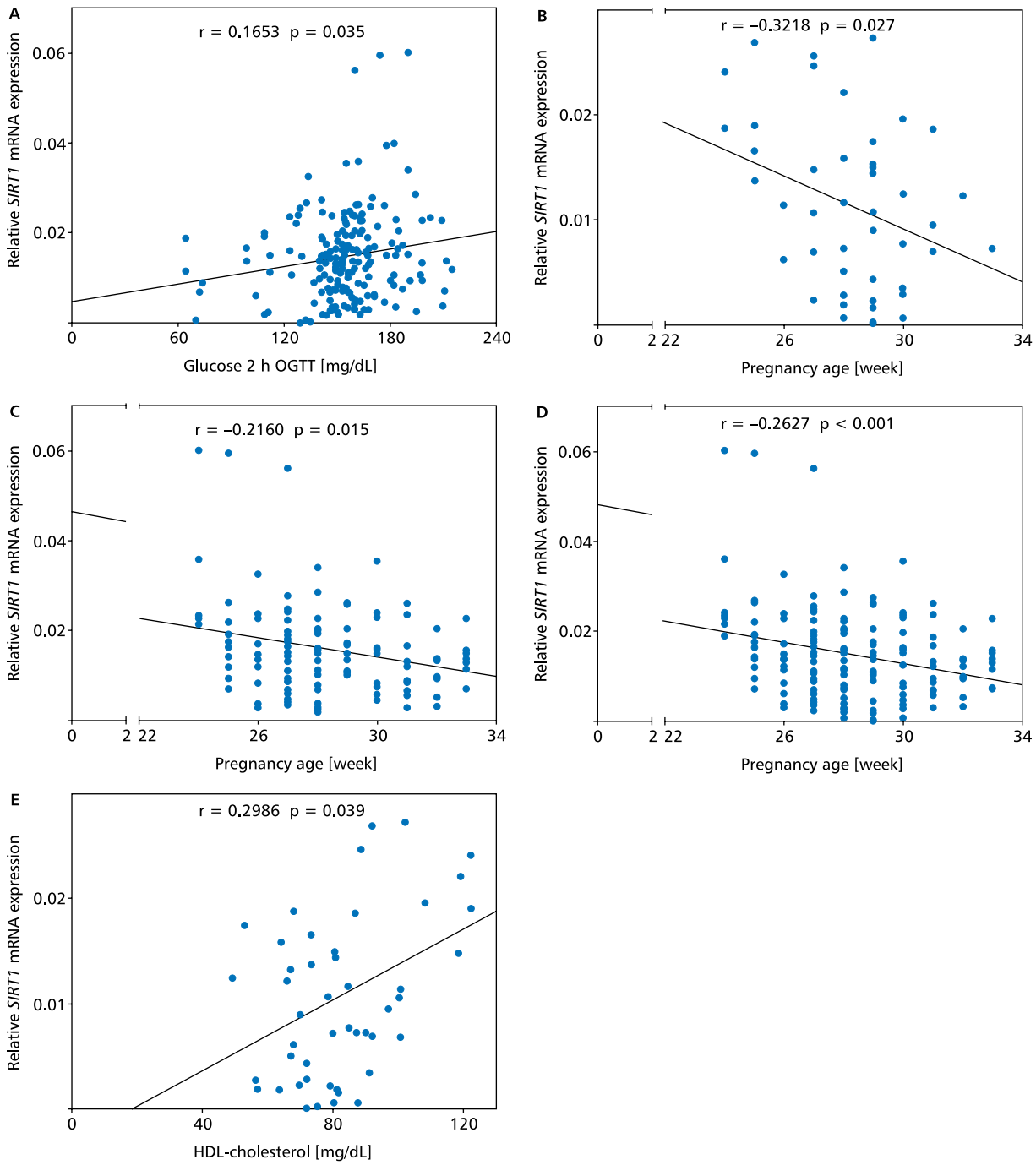

Figure 2. The correlations between leukocyte *SIRT1* mRNA expression and 2 h OGTT glucose plasma concentration in the entire study group (**A**), gestational age in the NGT and GDM groups (**B** and **C**) as well as in the whole study population (**D**), and plasma HDL-cholesterol level in the NGT controls (**E**)

the maternal organism to enhanced oxidative stress during diabetic pregnancy. Unfortunately, oxidative stress markers that could fully confirm this hypothesis have not been determined in this study. Therefore further studies are clearly needed in this field to completely elucidate the relationship of leukocyte SIRT1 with hyperglycemia-mediated oxidative stress in the GDM women in the third trimester of pregnancy.

Another interesting finding in the current study is the positive correlation of leukocyte *SIRT1* mRNA with plasma HDL-cholesterol in the NGT group. The plasma HDL-cholesterol level is currently believed to be a major determinant of susceptibility to coronary atherosclerosis since its concentration inversely correlated with atherosclerotic cardiovascular disease risk in humans [34]. It is well-known that anti-atherogenic HDL-cholesterol

properties stem from its anti-oxidant, anti-thrombotic, anti-inflammatory, vasodilatory, and endothelial repair activities as well as its capacity to mediate cellular cholesterol efflux through acting as primary acceptor, thereby promoting reverse cholesterol transport (RCT) from the artery wall and peripheral tissue to the liver [35]. The relationship of SIRT1 with HDL metabolism and RCT has been found. In this regard, SIRT1 has been identified as a positive regulator of liver X receptors (LXRs), belonging to the nuclear receptors superfamily, which modulate key genes involved in HDL metabolism and RCT, including the ATP-binding cassette transporter A1 (ABCA1) [36]. Consistent with this, SIRT1 knockout mice exhibited abnormal cholesterol homeostasis due to decreased ABCA1 expression resulting in a reduction of RCT as well as HDL-cholesterol [36]. Given the above, it is possible that the positive correlation of leukocyte *SIRT1* mRNA with plasma HDL-cholesterol concentration in the NGT group observed in our study might reflect the protective role of leukocyte *SIRT1* overexpression against atherosclerotic cardiovascular diseases in healthy pregnant women in the third trimester of pregnancy through an increase of HDL-cholesterol formation resulting, at least partially, from enhanced RCT. However, the precise mechanism responsible for such association remains to be elucidated in the future.

Another remarkable observation of this study is that leukocyte *SIRT1* expression correlated inversely with pregnancy age in both the GDM and NGT pregnant women. However, the causes and effects of this relationship are impossible to determine in the current study since no information exists on this topic in the literature. Thus, further research are required in this fields to explain why leukocyte *SIRT1* expression decreases with increasing gestational age of pregnant women with and without GDM. In this regard, changes in metabolic and endocrine factors should be considered since they play a central role in the course of normal and diabetic pregnancy.

Conclusions

To date, there is a paucity of data on SIRT1 and GDM. This study provides, for the first time, five novel valuable data information. First, leukocyte *SIRT1* mRNA was markedly increased in the GDM vs. NGT women in the third trimester of pregnancy, suggesting that GDM might affect changes in SIRT1 at its gene expression level. Second, leukocyte *SIRT1* gene expression was correlated positively with the plasma glucose concentration in the GDM women, implying that higher *SIRT1* expression could reflect the hyperglycemic state in diabetic patients. In this regard, the major limitation of our study was the lack of measurements of oxidative stress markers that would help to clarify the link between hyperglycemia-mediated oxidative stress and leukocyte *SIRT1* overexpression in the GDM women. Third, leukocyte *SIRT1* expression was correlated positively with HDL-cholesterol in the NGT pregnant women, suggesting that enhanced expression of this enzyme could have a beneficial effect on increased HDL-cholesterol production during normal pregnancy. Fourth, leukocyte *SIRT1* expression was correlated inversely with pregnancy age in both the GDM and NGT pregnant women. However, the establishment of the causes and effects of this association requires further studies. Fifth, leukocytes seem to be a convenient experimental model to detect changes in *SIRT1* expression and to assess the potential mechanisms involved in these alterations since there is a serious ethical problem linked to any invasive method used to obtain metabolic tissue samples from pregnant women.

Acknowledgements

This study was financially supported by the Medical University of Lodz (project no. 502-03/0-160-01/502-04-015 to I.T.).

Authors are grateful dr Małgorzata Bienkiewicz for helping in statistical analyses.

Statement of competing interests

The authors report no competing interests.

REFERENCES

1. Wojcik M., Mac-Marcjanek K., Wozniak L.A., Physiological and pathophysiological functions of SIRT1. Mini Rev. Med. Chem. 2009; 9: 386–394.
2. Picard F., Kurtev M., Chung N. i wsp. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 2004; 429: 771–776.
3. Rodgers J.T., Lerin C., Haas W., Gygi S.P., Spiegelman B.M., Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1 alpha and SIRT1. Nature 2005; 434: 113–118.
4. Bordone L., Motta M.C., Picard F. i wsp. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. PLoS Biol. 2006; 4: e31.
5. Gerhart-Hines Z., Rodgers J.T., Bare O. i wsp. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. EMBO J. 2007; 26: 1913–1923.
6. Nayagam V.M., Wang X., Tan Y.C. i wsp. SIRT1 modulating compounds from high-throughput screening as anti-inflammatory and insulin-sensitizing agents. J. Biomol. Screen 2006; 11: 959–967.
7. Yoshizaki T., Schenk S., Imamura T. i wsp. SIRT1 inhibits inflammatory pathways in macrophages and modulates insulin sensitivity. Am. J. Physiol. Endocrinol. Metab. 2010; 298: E419–E428.
8. Csiszar A., Smith K., Labinskyy N., Orosz Z., Rivera A., Ungvari Z. Resveratrol attenuates TNF-α-induced activation of coronary arterial endothelial cells: role of NF-κB inhibition. Am. J. Physiol. Heart Circ. Physiol. 2006; 291: H1694–H1699.
9. Yeung F., Hoberg J.E., Ramsey C.S. i wsp. Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J. 2004; 23: 2369–2380.
10. Coustan D.R., Lowe L.P., Metzger B.E., Dyer A.R.; International Association of Diabetes and Pregnancy Study Groups. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: paving

the way for new diagnostic criteria for gestational diabetes mellitus. Am. J. Obstet. Gynecol. 2010; 202: e1–e6.
11. Forsbach-Sánchez G., Tamez-Peréz H.E., Vazquez-Lara J. Diabetes and pregnancy. Arch. Med. Res. 2005; 36: 291–299.
12. Metzger B.E., Buchanan T.A., Coustan D.R. i wsp. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care 2007; 30 (supl. 2): S251–S260.
13. Lappas M., Hiden U., Desoye G., Froehlich J., Hauguel-de Mouzon S., Jawerbaum A. The role of oxidative stress in the pathophysiology of gestational diabetes mellitus. Antioxid Redox Signal. 2011; 15: 3061–3100.
14. Chen X., Scholl T.O. Oxidative stress: changes in pregnancy and with gestational diabetes mellitus. Curr. Diab. Rep. 2005; 5: 282–288.
15. Salmi A.A., Zaki N.M., Zakaria R., Nor Aliza A.G., Rasool A.H. Arterial stiffness, inflammatory and pro-atherogenic markers in gestational diabetes mellitus. Vasa 2012; 41: 96–104.
16. López-Tinoco C., Roca M., García-Valero A. i wsp. Oxidative stress and antioxidant status in patients with late-onset gestational diabetes mellitus. Acta Diabetol. 2013; 50: 201–208.
17. Altinova A.E., Toruner F., Bozkurt N. i wsp. Circulating concentrations of adiponectin and tumor necrosis factor-alpha in gestational diabetes mellitus. Gynecol. Endocrinol. 2007; 23: 161–165.
18. Chen D., Xia G., Xu P., Dong M. Peripartum serum leptin and soluble leptin receptor levels in women with gestational diabetes. Acta Obstet. Gynecol. Scand. 2010; 89: 1595–1599.
19. Ranheim T., Haugen F., Staff A.C., Braekke K., Harsem N.K., Drevon C.A. Adiponectin is reduced in gestational diabetes mellitus in normal weight women. Acta Obstet. Gynecol. Scand. 2004; 83: 341–347.
20. Song R., Xu W., Chen Y., Li Z., Zeng Y., Fu Y. The expression of Sirtuins 1 and 4 in peripheral blood leukocytes from patients with type 2 diabetes. Eur. J. Histochem. 2011; 55: e10.
21. Kitada M., Koya D. SIRT1 in Type 2 Diabetes: Mechanisms and Therapeutic Potential. Diabetes Metab. J. 2013; 37: 315–325.
22. Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.A., Treacher D.F., Turner R.C. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419.
23. Katz A., Nambi S.S., Mather K. i wsp. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J. Clin. Endocrinol. Metab. 2000; 85: 2402–2410.
24. Wojcik M., Zieleniak A., Mac-Marcjanek K., Wozniak L.A., Cypryk K. The elevated gene expression level of the A2B adenosine receptor is associated with hyperglycemia in women with gestational diabetes mellitus. Diabetes Metab. Res. Rev. 2013; doi: 10.1002/dmrr.2446.
25. Livak K.J., Schmittgen T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402–408.
26. Qiao L., Shao J. SIRT1 regulates adiponectin gene expression through Foxo1-C/enhancer-binding protein alpha transcriptional complex. J. Biol. Chem. 2006; 281: 39915–39924.
27. Sun C., Zhang F., Ge X. i wsp. SIRT1 improves insulin sensitivity under insulin- resistant conditions by repressing PTP1B. Cell Metab. 2007; 6: 307–319.
28. de Kreutzenberg S.V., Ceolotto G., Papparella I. i wsp. Down-regulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: potential biochemical mechanisms. Diabetes 2010; 59: 1006–1015.
29. Chen D., Bruno J., Easlon E. i wsp. Tissue-specific regulation of SIRT1 by calorie restriction. Genes Dev. 2008; 22: 1753–1757.
30. Wozniak L.A., Cypryk K., Wojcik M. Molecular mechanisms of diabetes prevention by structurally diverse antioxidants (Chapter 25). W: Nutritional and therapeutic interventions for diabetes and metabolic syndrome (Ed. Debasis Bagchi and Nair Sreejayan). Elsevier, San Diego (USA) 2012, 315–330.
31. Hasegawa K., Wakino S., Yoshioka K. i wsp. Sirt1 protects against oxidative stress-induced renal tubular cell apoptosis by the bidirectional regulation of catalase expression. Biochem. Biophys. Res. Commun. 2008; 372: 51–56.
32. Alcendor R.R., Gao S., Zhai P. i wsp. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circ. Res. 2007; 100: 1512–1521.
33. Kitamura Y.I., Kitamura T., Kruse J.P. i wsp. FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction. Cell Metab. 2005; 2: 153–163.
34. Boden W.E. High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the Veterans Affairs High-Density Lipoprotein Intervention Trial. Am. J. Cardiol. 2000; 86: 19L–22L.
35. Kontush A., Chapman M.J. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacol. Rev. 2006; 58: 342–374.
36. Li X., Zhang S., Blander G., Tse J.G., Krieger M., Guarente L. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. Mol. Cell. 2007; 28: 91–106.