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ORIGINAL PAPER

Glyoxalase 1 and glyoxalase 2 activities in blood and neuronal tissue samples from experimental animal models of obesity and type 2 diabetes mellitus

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Abstract The glyoxalase enzymes catalyse the conversion of reactive glucose metabolites into non-toxic products as a part of the cellular defence system against glycation. This study investigated changes in glyoxalase 1 and glyoxalase 2 activities and the development of diabetic complications in experimental animal models of obesity (Zucker fa/fa rats) and type 2 diabetes mellitus (Goto-Kakizaki rats). In contrast to Zucker rats, in Goto-Kakizaki rats the glyoxalase 1 activities in brain, spinal cord and sciatic nerve tissues were significantly reduced by 10, 32 and 36 %, respectively. Lower glyoxalase 1 activity in the neuronal tissues was associated with a higher blood glucose concentration and impaired endothelium-dependent relaxation to acetylcholine in aortic rings in Goto-Kakizaki rats. This study provides evidence for disturbed neuronal glyoxalase 1 activity under conditions of hyperglycaemia in the presence of impaired endothelium-dependent relaxation and cognitive function.

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Introduction

Diabetes mellitus is the most common metabolic disease and is primary defined by the level of hyperglycaemia. Downstream consequences of increased glucose concentration include multiple pathophysiological processes, such as protein glycation and inflammation, which can lead to biochemical dysfunction associated with the chronic development of microvascular damage [1–3]. Glycation leading to advanced glycation end-product (AGE) formation and accumulation has been associated with aging [4] and is found to be increased in diabetes [5].

The glyoxalase system, which consists of glyoxalase 1 (EC 4.4.1.5, Glo1) and glyoxalase 2 (EC 3.1.2.6, Glo2), is critical for the detoxification of reactive dicarbonyls (mainly methylglyoxal), which are potent precursors of AGEs [6]. Previous studies have shown that the glyoxalase system is modified in the tissues of streptozotocin-induced diabetic rats [7], and altered blood Glo1 activity is associated with late diabetic complications [8–10]. Moreover, it has been shown that enhancement of the glyoxalase pathway along with a decrease in methylglyoxal-dependent protein glycation prevents the development of several indices of late complications in diabetic mice [11]. However, the factors responsible for the variable susceptibility to diabetic complications and the role of the glyoxalase system in the development of vascular complications in diabetes and obesity are not clear.

Both glucotoxicity and lipotoxicity are proposed to play a role in the development of late diabetic complications. Hyperglycaemia leads to mitochondrial dysfunction and the

activation of stress pathways [1, 12], while free fatty acid-mediated lipotoxicity disturbs glucose uptake and metabolism, induces insulin resistance and damages β-cell function [13, 14]. Several animal models are available for the experimental delineation of the detrimental effects of glucotoxicity and lipotoxicity. Leptin-deficient Zucker fa/fa [15] rats are characterised by obesity, insulin resistance, hyperinsulinaemia, and dyslipidaemia, and they develop late diabetic complications as a consequence of obesity-related processes [16]. It has been shown that oxidative stress caused by lipid peroxidation in the absence of hyperglycaemia is involved in tissue lesions and functional impairments in obese Zucker rats [17]. The Goto-Kakizaki rat is a spontaneous type 2 diabetic model [18] that shows the main features of the metabolic, hormonal, and vascular disorders usually described in type 2 diabetes mellitus [19]. The comparative activities of Glo1 and Glo2 in relation to the development of micro- and macrovascular complications of diabetes in Zucker fa/fa and Goto-Kakizaki rats have not been studied.

Clinical studies have shown that pre-diabetes is an intermediate condition associated with specific microvascular alterations, including neuropathy [20, 21] and cognitive impairment [22]. To investigate the early events of the development of diabetic micro- and macrovascular complications before their clinical onset, in this study, we used experimental animal models of obesity (Zucker fa/fa rats) and type 2 diabetes mellitus (Goto-Kakizaki rats) and monitored the change in blood Glo1 and Glo2 activities and the development of diabetic complications during a 16-week period. At the end of the experiment, samples of the brain, spinal cord, and sciatic nerve tissue were collected, and Glo1 and Glo2 activities and expression were measured. In addition, peripheral thermal and mechanical pain perception, working memory in spontaneous alternation, and vascular reactivity in isolated aortic rings were evaluated as measures of the functional manifestations of vascular complications in diabetes.

Materials and methods

Chemicals

Tris (hydroxymethyl) aminomethane, potassium dihydrogen phosphate, sodium hydrogen phosphate, sodium chloride, potassium chloride, calcium chloride, magnesium sulphate, sodium bicarbonate and EDTA (ethylenediaminetetraacetic acid) were purchased from Acros Organics (Geel, Belgium). S-D-lactoylglutathione, ~40 % methylglyoxal solution, reduced gluthatione (GSH), glucose, phenylmethylsulphonylfluoride (PMSF), diamide, pepstatin, leupeptin, aprotinin, noradrenalin and acetylcholine were obtained from Sigma-Aldrich (Steinheim, Germany).

Animals and experimental design

All animal care and experimental procedures were performed in accordance with the guidelines of the European Community, local laws and policies, and were approved by the Latvian Animal Protection Ethical Committee, Food and Veterinary Service, Riga, Latvia. Twelve male Zucker fa/fa, Zucker lean (Charles River Laboratories, France), Goto-Kakizaki and Wistar-Kyoto rats (Taconic Farms, USA) were purchased at 7 weeks of age, 1 week prior to the beginning of the experiments. The animals were housed under standard conditions (21–23 °C, 12-h light–dark cycle) with unlimited access to food (R3 diet; Lactamin, Sweden) and water. Zucker lean and Wistar rats were used as the non-diabetic controls to evaluate the progression of diabetes in Zucker fa/fa and Goto-Kakizaki rats, respectively. Blood samples were collected from the tail vein of the experimental animals at 8, 12, 16, 20 and 24 weeks of age.

Lipid profile, HbA_{1C}, plasma glucose and insulin concentrations and intraperitoneal glucose tolerance test

For biochemical analysis, blood was collected in 5 mM EDTA/5 mM diamide- and protease inhibitor (0.1 μM PMSF, 1 μM pepstatin, 10 μM leupeptin, 1.5 μM aprotinin)-containing tubes and centrifuged, and the obtained plasma was stored at −80 °C until analysis. Triglycerides and plasma glucose were measured by IL Laboratories (Lexington, USA) enzymatic kits. Free fatty acids (FA) were determined using commercially available enzymatic kit from Wako (Neuss, Germany). The plasma insulin concentration was determined with a Sensitive Rat Insulin RIA kit (Millipore, Billerica, USA). HbA_{1C} in whole blood samples was measured by high-pressure liquid chromatography (Primus, Kansas City, USA) in an accredited diagnostic laboratory.

The diabetic and control rats were subjected to fasting overnight to perform the glucose tolerance test. For the glucose tolerance experiments, a glucose solution (1 g/kg of body weight) was administered intraperitoneally, and blood samples were then drawn from the tail vein at 0 (fasting), 5, 15, 30, 45, 60, 120 and 240 min.

Measurement of Glo1 and Glo2 activity in the blood samples

Blood samples used for the measurement of Glo1 and Glo2 activities were collected in EDTA-containing tubes, and whole blood lysates were prepared for assay shortly after sampling. A Glo1 activity assay and blood lysate preparation was performed according to a previously described

method [23, 24] by monitoring the increase in absorbance at 240 nm due to the formation of S-D-lactoylglutathione for 5 min in a 96-well UV-transparent plate (Greiner Bio-One, Germany). The concentration of hemithioacetal used in assay is 1.33 mM according to Van der Jagt et al. [25]. Glo2 activity was assayed using spectrophotometry according to a previously described method [24] by monitoring the decrease in absorbance at 240 nm due to the hydrolysis of S-D-lactoylglutathione for 5 min. The initial concentration of S-D-lactoylglutathione was 500 μM in a 50 mM Tris–HCl buffer solution at a pH of 7.4. The enzyme activities were calculated based on standard curve of S-D-lactoylglutathione (99 %, Cat.No.L7140; Sigma-Aldrich). The Glo1 activity in the whole blood lysates was defined as the formation of μmol S-D-lactoylglutathione per min per g of haemoglobin. The Glo2 activity in the whole blood lysates was defined as the hydrolysis of μmol S-D-lactoylglutathione per min per g of haemoglobin.

Measurement of Glo1 and Glo2 activity in tissue homogenates

At the end of the experiment, tissue samples from the brains, spinal cords, and sciatic nerves from 24-week-old diabetic and control rats were collected. The tissues were stored at −80 °C until analysis. Tissue homogenates were prepared as described by Phillips et al. [7] with some modifications. Briefly, tissue was homogenized with a Cole Parmer 130-W ultrasonic processor set at 20 kHz for 30 s in an ice-cold 10 mM sodium phosphate buffer, pH 7.4 at w/v ratio of 1:10 and centrifuged at 20,000*g* for 10 min at +4 °C. The supernatant was used to measure Glo1 and Glo2 activity as described previously. The protein concentration was determined using the Lowry assay. The Glo1 activity in tissue homogenates was defined as the formation of μmol S-D-lactoylglutathione per min per g of protein. The Glo2 activity in tissue homogenates was defined as the hydrolysis of μmol S-D-lactoylglutathione per min per g of protein.

Tail-flick and paw pressure test

The tail-flick test was performed as described previously by Liepinsh et al. [26]. Thermal pain sensitivity was measured as the change in reaction latency to noxious thermal stimuli assessed by a tail-flick apparatus (Model DS20; Ugo Basile, Italy). The cut-off time was fixed at 20 s to avoid damage to the tail.

The paw pressure was determined using an analgesy meter (Ugo Basile), and pressure was applied to the right and left hind foot. The force applied (*g*) increased at a constant rate until foot withdrawal occurred. The test was repeated five times at approximately 5-min intervals on each animal, and the mean value was calculated. This was the measurement recorded as the mechanical nociceptive threshold.

Spontaneous alternation behaviour in the Y-maze test

Working memory performance was assessed by recording spontaneous alternation behaviour in the Y-maze as described previously [27]. The rats were placed individually at the end of one arm in a symmetrical Y-shaped runway (arm length 40 cm, width 11 cm, height 28 cm) and allowed to explore the maze for 5 min. An alternation was defined as entries into all three arms on consecutive occasions. The total number and sequence of arm entries were manually recorded and the percentage of alternation behaviour was calculated [28].

Isolated aortic ring experiments

The thoracic aorta was excised, immersed in ice-cold Krebs–Henseleit (K–H) buffer (content in mM: NaCl 118, KCl 4.75, $CaCl_2$ 1.8, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.18, glucose 11.0) and cleaned of fatty and connective tissue. The aorta was cut into aortic rings that were 3 mm in length and mounted between two stainless steel hooks in oxygenated K–H buffer (pH 7.4, 37 °C). The passive tension was fixed at 20 mN. After a period of equilibration (0.5 h), the maximal contractility was assessed by adding 80 mM KCl. Then, the aortic rings were precontracted with noradrenaline to sub-maximal contractility (60–80 % of maximal contraction), and the function of the endothelium was assessed by adding cumulative doses (10^{-10}–10^{-5} M) of acetylcholine.

Isolation of RNA and quantitative RT-PCR analysis

Tissue for RT-PCR analysis was immediately removed and frozen in liquid nitrogen for subsequent RNA isolation. Total RNA from brain, spinal cord, and sciatic nerve tissue were isolated using the TRI Reagent (Sigma, St. Louis, USA) according to the manufacturer's protocol. The quality and quantity of the extracted total RNA were examined, and first-strand cDNA was synthesised using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA) following the manufacturer's instructions. Quantitative RT-PCR analysis for GLO1 (F—5′-ATTTGGCCACATTGGGATTGC-3′, R—5′-TTCAATCCAGTAGCCATCAGG-3′) and GLO2 (F—5′-TGACACATTGTTTGTTGCTG-3′, R—5′-CGGTCTTCTCCTTCACTCTC-3′) was performed by mixing synthesised cDNA, primers, and SYBR® Green Master Mix (Applied Biosystems) and run in the Applied Biosystems Prism 7500 according to the manufacturer's protocol.

Transcript levels for the constitutive housekeeping gene product β-actin were quantitatively measured for each sample, and PCR data were reported as the number of transcripts per number of β-actin mRNA molecules. To avoid genomic DNA contamination, the primers were also designed to span an intron.

Data analysis

The data are presented as mean ± SEM. Statistically significant differences in the mean values for the Zucker or Goto-Kakizaki rats compared to the appropriate control animals were tested using the Student's t test. The paired t test was used to compare the time-dependent changes in Glo1 and Glo2 activity compared to the baseline. Spearman's correlation analysis was used to examine the relationship between neuronal tissue Glo1 activity and blood biochemical parameters or vascular complications. To compare the response of aortic rings to noradrenalin or acetylcholine, a two-way ANOVA with the Bonferroni post hoc test was performed. The differences were considered to be significant when $p < 0.05$. The data were analysed using Graph Pad Prism 3.0 statistical software (Graph Pad, USA).

Results

Plasma glucose, glucose tolerance test, and insulin and lipid levels

Goto-Kakizaki rats developed mild hyperglycaemia, and, at the end of the experiment, the plasma glucose level was approximately 1.5-fold greater in the fasted and fed states compared to age-matched Wistar control rats, while Zucker fa/fa rats had an increase in the plasma glucose concentration of 12 % only in the fasted state. The fed-state plasma glucose levels in the Zucker fa/fa rats were similar to those of the Zucker lean rats (Fig. 1a). The concentration of HbA_{1C} in the Goto-Kakizaki and Zucker fa/fa rats was 8.4 ± 0.1 and 6.7 ± 0.2 %, respectively, and significantly differed from the control animals (Table 1). The integrated areas under the curve (AUCs) for glucose were calculated from the glucose tolerance test results. The blood glucose levels in the Goto-Kakizaki and Zucker fa/fa rats at all time points were significantly higher compared to the control rats, and the AUC was 3- and 2-fold greater, respectively (Table 1). The fasting plasma insulin concentration in the Goto-Kakizaki rats was 1.5-fold greater compared to control rats, but the fed plasma insulin concentrations were similar in the Goto-Kakizaki and control rats. The Zucker fa/fa rats had a marked 10- and 8-fold increase in insulin concentration in the fed and fasted states, respectively, compared to the lean rats (Table 1).

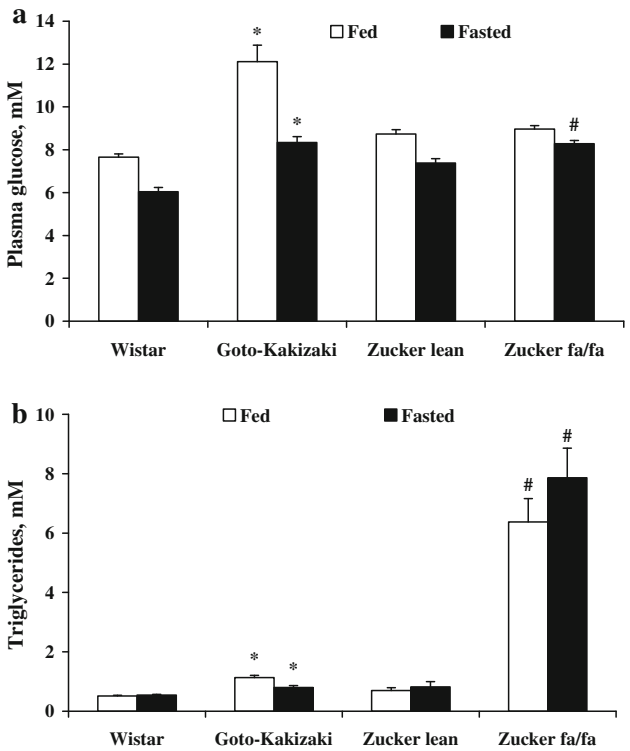

Fig. 1 Plasma glucose (**a**) and triglycerides (**b**) concentrations in Wistar, Goto-Kakizaki, Zucker lean and Zucker fa/fa rats at 24 weeks of age. The data are presented as the mean ± SEM of at least 11 animals. $^{*}p < 0.05$ compared to the Wistar control. $^{\#}p < 0.05$ compared to the Zucker lean control

As expected, the Zucker fa/fa rats developed hyperlipidaemia, and the plasma triglyceride and FA levels in the fed state were 9- and 2-fold higher, respectively, compared to the lean control rats (Fig. 1b; Table 1). The Goto-Kakizaki rats had a significant 2- and 3-fold increase in plasma triglyceride and FA levels in the fed state and a 48 and 20 % increase in the fasted state, respectively, compared to the Wistar rats (Fig. 1b; Table 1).

Glo1 and Glo2 activities and expression

Glo1 activity in the blood samples was 3-fold higher compared to that of Glo2 (Fig. 2a, b). Blood Glo1 and Glo2 activity in the Goto-Kakizaki rats at the start of the experiment at 8 weeks of age was significantly increased by 12 and 15 %, respectively, compared to the age-matched Wistar rats, but after 16 weeks, this difference decreased to 5 and 9 %, respectively (Fig. 2a, b). However, the Glo1 activity in the neuronal tissue (brain, spinal cord and sciatic nerve) of the Goto-Kakizaki rats was significantly reduced by 10, 32 and 36 %, respectively (Fig. 3a). Glo2 activity in the Goto-Kakizaki rats was significantly reduced only in the cortex and spinal cord tissue by 8 and

Table 1 Biochemical parameters at the end of the study

	Wistar	Goto-Kakizaki	Zucker lean	Zucker fa/fa
Fed state				
Insulin (ng/ml)	2.15 ± 0.43	3.67 ± 0.86	1.44 ± 0.18	11.39 ± 1.31#
Free fatty acids (mM)	0.37 ± 0.03	1.05 ± 0.13*	0.54 ± 0.03	0.91 ± 0.05#
HbA_{1C} (%)	7.59 ± 0.33	8.39 ± 0.14*	5.75 ± 0.37	6.74 ± 0.22#
Fasted state				
Insulin (ng/ml)	0.31 ± 0.03	0.48 ± 0.07*	0.50 ± 0.04	5.01 ± 0.75#
Free fatty acids (mM)	1.22 ± 0.06	1.47 ± 0.08*	1.23 ± 0.07	1.28 ± 0.07
Glucose tolerance test (AUC)	1473 ± 110	3932 ± 221*	812 ± 62	1542 ± 131#

The values are presented as the mean ± SEM of at least 11 animals

* $p < 0.05$ compared to the Wistar control

# $p < 0.05$ compared to the Zucker lean control

15 %, respectively. Glo2 activity was not significantly changed in sciatic nerve (Fig. 3b). GLO1 and GLO2 expression in the brain, spinal cord and sciatic nerve tissue of 24-week-old Goto-Kakizaki rats compared to the controls was not altered (data not shown).

In the Zucker fa/fa rats, the blood and neuronal Glo1 activity was similar to that of the control animals (Figs. 2c, 3c). Glo2 activity in the blood samples was increased on average from 5 to 20 % during the 16-week period (Fig. 2d). Glo1 and Glo2 activities in the Zucker fa/fa rat tissue were unchanged or had a tendency to be increased compared to the lean Zucker rats. Although Glo2 activity in the blood samples at 24 weeks of age was elevated by 16 % compared to the lean controls, only in the hypothalamus was Glo2 activity significantly increased (Figs. 2d, 3d).

Vascular relaxation, peripheral pain perception and spontaneous alternation behaviour

Endothelium-dependent relaxation to acetylcholine in the aortic rings in the Goto-Kakizaki and Zucker fa/fa rats was significantly impaired compared to the controls (Fig. 4a, b). In addition, our results show that endothelial dysfunction was more pronounced in the Goto-Kakizaki rats compared to the Zucker fa/fa rats. The maximal aortic ring response to acetylcholine in the Goto-Kakizaki and Zucker fa/fa rats compared to the control animals were decreased by 48 and 13 %, respectively (Fig. 4a, b).

The tail-flick, paw-pressure and Y-maze tests were performed at 8, 16 and 24 weeks. The results indicated no changes in peripheral thermal and mechanical pain perception until the 24th week in the Zucker fa/fa and Goto-Kakizaki rats compared to the control animals (data not shown). In the Y-maze test, the spontaneous alternation behaviour in the Goto-Kakizaki and Zucker fa/fa rats compared to the control animals at 24 weeks of age were significantly reduced by 39 and 19 %, respectively (Fig. 5a, b). The working memory in the Goto-Kakizaki and Zucker fa/fa rats at 8 and 16 weeks of age was similar to that of the control animals.

Correlation of neuronal tissue Glo1 activity with biochemical parameters and vascular complications

To detect if changes in Glo1 activity in the rat brain, spinal cord and sciatic nerve tissue were associated with blood biochemical parameters or vascular complications, Spearman correlation analysis was performed. Correlation analysis of the study data showed that, in the Goto-Kakizaki rats, hypothalamus, spinal cord and sciatic nerve, Glo1 activity negatively correlated with the plasma glucose and triglyceride levels (Table 2). A strong positive correlation with the maximal endothelial relaxation was found in all neuronal tissues from the Goto-Kakizaki rats (Table 2). There were no significant associations between blood and tissue Glo1 and Glo2 activities in Goto-Kakizaki rats, but a moderate positive correlation between blood Glo1 and Glo2 activity and Glo1 activity in the Zucker rat brain cortex was detected (data not shown). There were no significant associations between the spontaneous alterations in Y-maze test and Glo1 activities in neuronal tissues of Goto-Kakizaki and Zucker rats (Table 2). No significant associations between tissue Glo1 activity and other measured parameters in the Zucker rats were found.

Discussion

The primary finding of this study was that in 24-week-old Goto-Kakizaki rats, which exhibited mild hyperglycaemia and marked glucose intolerance, there were no major alterations in Glo1 and Glo2 activities in the blood samples, but the Glo1 and Glo2 activities in neuronal tissues was significantly reduced. Zucker fa/fa rats developed hypertriglyceridaemia, obesity and lipid overload-induced insulin resistance, but Glo1 activity in the blood samples and neuronal tissue of obese Zucker rats was similar to that of the control animals. The overall changes in Glo2 activity followed the pattern of changes in the activity of Glo1, which functions as the most important methylglyoxal-detoxifying enzyme [28]. Additionally, our data showed

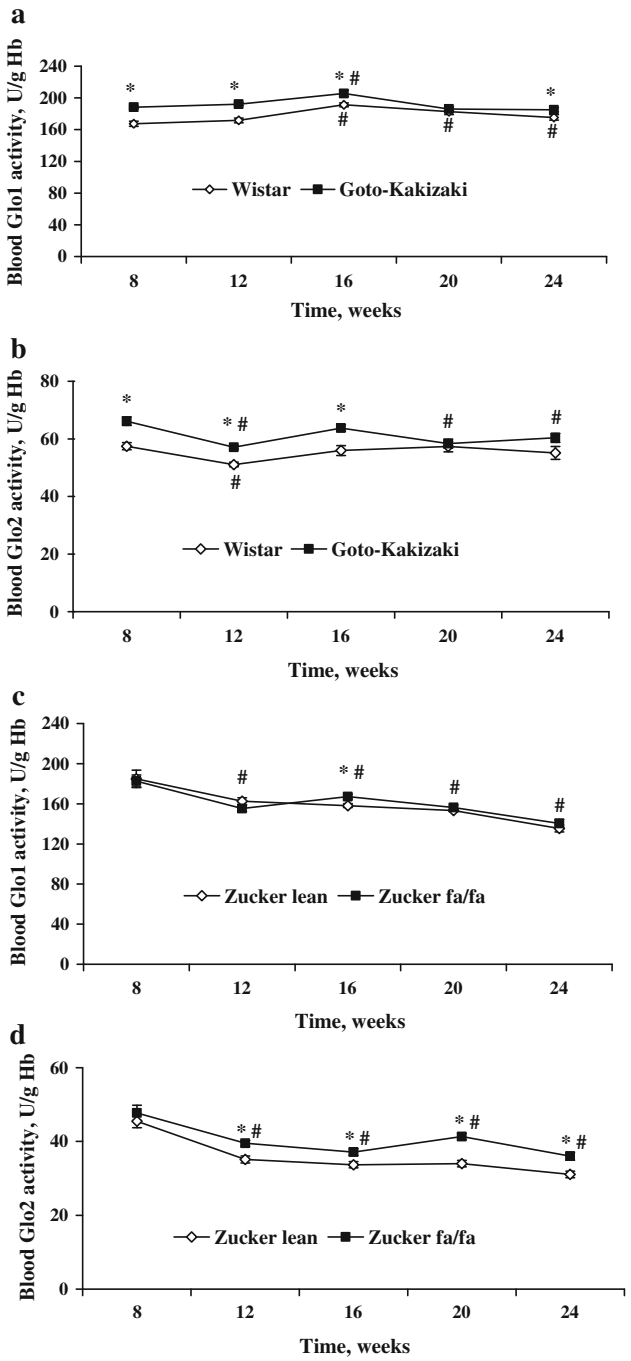

Fig. 2 Glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2) activities in blood. Changes in Glo1 and Glo2 activity in blood from Wistar, Goto-Kakizaki (**a**, **b**) and Zucker fa/fa and lean (**c**, **d**) rats from 8 to 24 weeks of age. The Glo1 activity in the whole blood lysates was defined as the μmol of S-D-lactoylglutathione formed per min per g of haemoglobin. The Glo2 activity in the whole blood lysates was defined as μmol of S-D-lactoylglutathione hydrolysed per min per g of haemoglobin. The data are presented as the mean ± SEM of at least 11 animals. $^{*}p < 0.05$ compared to the control group, $^{\#}p < 0.05$ compared to 8 weeks in the respective group

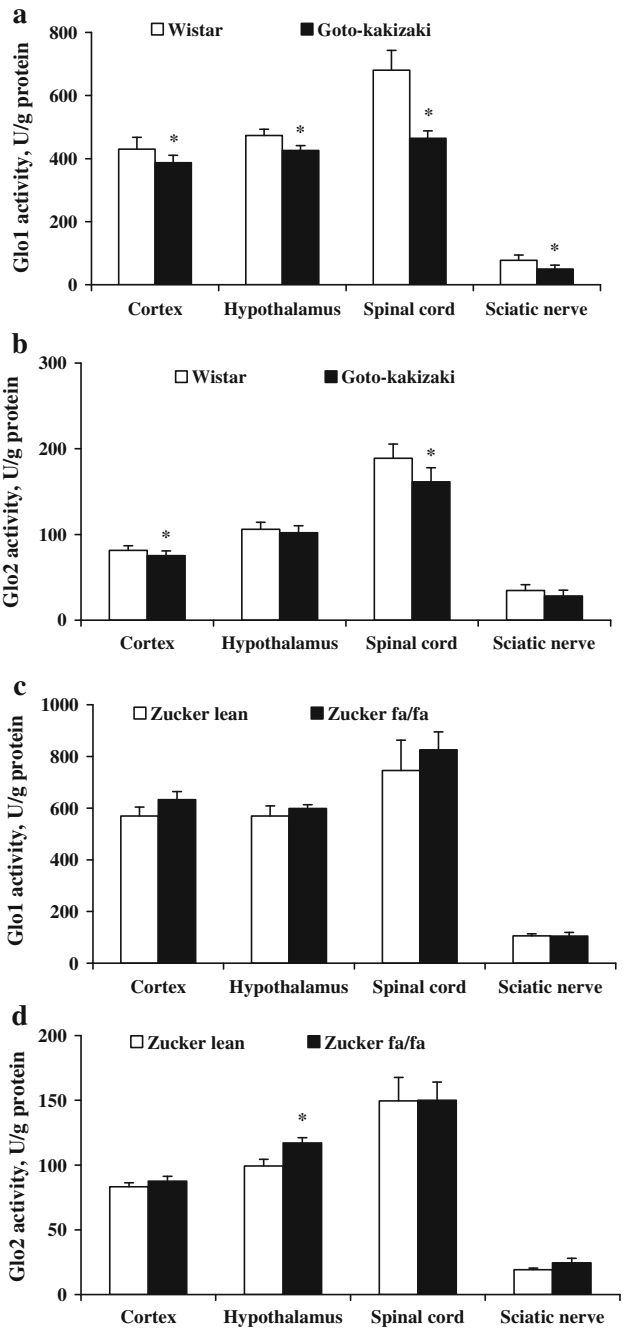

Fig. 3 Glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2) activities in neuronal tissues. Glo1 and Glo2 activity in the cortex, hypothalamus, spinal cord and sciatic nerve tissues from Wistar, Goto-Kakizaki (**a**, **b**) and Zucker fa/fa and lean (**c**, **d**) rats at 24 weeks of age. Non-diabetic rats (*white bars*), diabetic rats (*black bars*). The Glo1 activity in tissue homogenates was defined as the μmol of S-D-lactoylglutathione formed per min per g of protein. The Glo2 activity in tissue homogenates was defined as μmol of S-D-lactoylglutathione hydrolysed per min per g of protein. The data are presented as the mean ± SEM of at least 6 animals. $^{*}p < 0.05$ compared to the control group

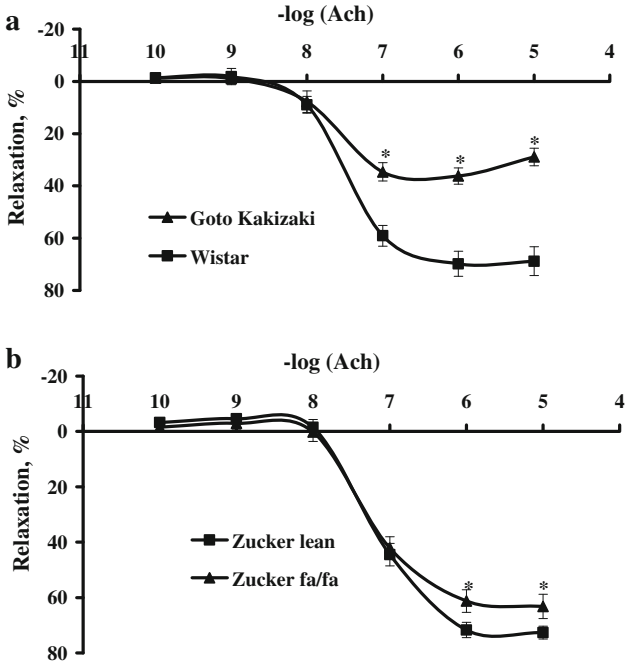

Fig. 4 Endothelium-dependent relaxation of isolated aortic rings in Wistar, Goto-Kakizaki, Zucker lean and Zucker fa/fa rats. Concentration–response curve of acetylcholine (10^{-10}–10^{-5}M) in isolated aortic rings of Wistar and Goto-Kakizaki (**a**) and Zucker lean and fa/fa (**b**) rats at 24 weeks of age. The data are presented as the mean % of relaxation ± SEM of at least 11 animals. *$p < 0.05$ compared to the control group

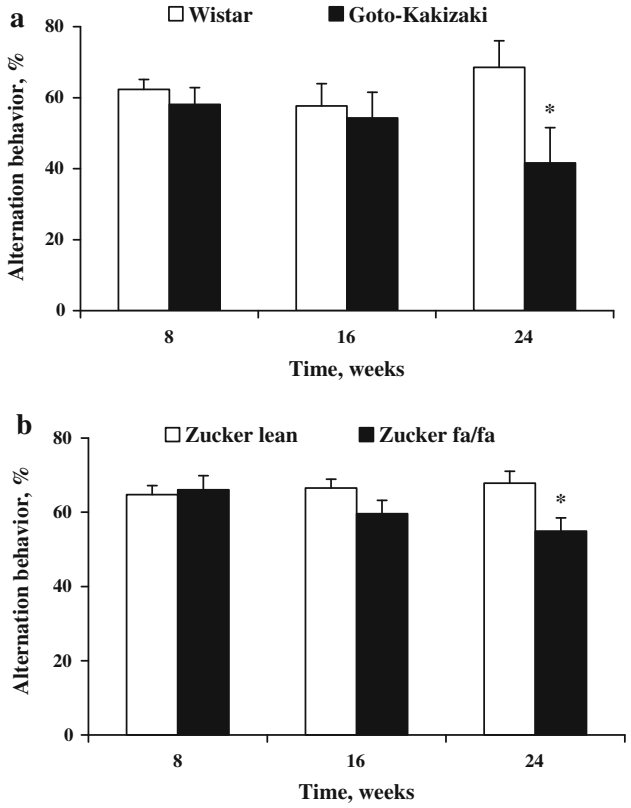

Fig. 5 Spontaneous alternation behaviour in Wistar, Goto-Kakizaki, Zucker lean and Zucker fa/fa rats. The spontaneous alternation behaviour in the Y-maze test in Wistar and Goto-Kakizaki (**a**) and Zucker lean and fa/fa (**b**) rats at 8, 16, and 24 weeks of age. Non-diabetic rats (*white bars*), diabetic rats (*black bars*). The data are presented as the mean % of alternation behaviour ± SEM of at least 11 animals. *$p < 0.05$ compared to the control group

that, in Goto-Kakizaki and Zucker rats, Glo1 activity in blood and neuronal tissues was 3- to 4-fold higher compared to Glo2 activity. Our measurements of enzyme activities in Goto-Kakizaki rats are in line with previous studies, which have shown that Glo1 and Glo2 activities in erythrocytes of streptozotocin-induced diabetic rats were not altered [7, 29]. However, the Glo1 activities in sciatic nerve and brain tissue of streptozotocin-induced diabetic rats were not different from control animals [7, 30], which were of younger age than those used in the present study. To our knowledge, the only study thus far that has investigated the glyoxalase system in an experimental model of obesity found that Glo2 activity was significantly increased in erythrocytes of obese (ob/ob) mice [31], similar to the obese Zucker rats in our study. However, while in our study Glo1 activity in blood of obese Zucker rats was not different compared to lean rats, the Glo1 activity was decreased in ob/ob mice at a similar age [31]. Thus, the observed changes in Glo1 and Glo2 activities in diabetic animals vary depending on experimental model, age and duration of diabetes. Correlation analysis of the study data showed that, in Goto-Kakizaki rats, Glo1 activity in the hypothalamus, spinal cord and sciatic nerve negatively correlated with plasma glucose and triglyceride levels. Thus, in Goto-Kakizaki rats, blood Glo1 activity was less sensitive to hyperglycaemia than neuronal Glo1. The model of hypertriglyceridaemia did not result in any significant changes in Glo1 activity.

Results from previous studies have demonstrated that altered blood Glo1 activity is associated with the development of late diabetic complications [8–10]. Impaired endothelium-dependent relaxation in conductance and resistance blood vessels has been reported before in Zucker fa/fa [32, 33] and Goto-Kakizaki rats [34, 35]. It has been shown recently that hyperglycaemia-induced impairment of endothelium-dependent vasorelaxation can be significantly improved by GLO1 overexpression [36]. Our results provide evidence for preserved endothelial function in diabetic Goto-Kakizaki rats with higher neuronal Glo1 activity. Thus, in our study, impaired endothelium-dependent vasodilatation in the aortic rings of both rat strains was observed at 24 weeks of age. We found a positive correlation between Glo1 activity in neuronal tissues and endothelial function in the aortic rings from Goto-Kakizaki rats, but not with acetylcholine-induced maximal relaxation of the aortic rings from Zucker rats. Moreover, we did

Table 2 Correlations of the biochemical parameters and vascular complications with glyoxalase 1 (Glo1) activity in various tissue samples of Wistar and Goto-Kakizaki rats

	Hypothalamus Glo1 activity (U/g protein)		Spinal cord Glo1 activity (U/g protein)		Sciatic nerve Glo1 activity (U/g protein)	
	r	*p* value	*r*	*p* value	*r*	*p* value
Plasma glucose (mM)	−0.76	0.01	−0.78	0.01	−0.79	0.01
Insulin (ng/ml)	−0.59	0.05	−0.51	ns	−0.47	ns
HbA_{1C} (%)	−0.67	0.02	−0.69	0.01	−0.52	ns
Triglycerides (mM)	−0.63	0.03	−0.81	0.01	−0.81	0.01
Maximal endothelium-dependent relaxation (%)	0.78	0.01	0.74	0.01	0.75	0.01
Alternation behaviour (%)	0.10	ns	0.27	ns	0.33	ns

Values are presented as Spearman correlation coefficients (*r*)

not find any relationship between blood Glo1 activity and endothelial function in either rat strain. Thus, it can be concluded that blood Glo1 activity does not reflect the functional status of vascular endothelium in experimental models of type 2 diabetes or metabolic syndrome. The measurement of blood Glo1 activity might be a useful approach to study late complications; however, our data from experimental models show that this activity does not reflect early disturbances in vascular reactivity.

No changes in peripheral thermal and mechanical pain perception were observed in the Goto-Kakizaki or obese Zucker fa/fa rats compared to the respective control animals, even though the Glo1 activity in the neuronal tissues was decreased by 10–36 %. Taking into account the impaired vascular reactivity in both rat strains, this indicates an early stage of diabetes and obesity-related complications. It has been reported before that changes in vascular reactivity to acetylcholine in obese Zucker rats precedes impaired neural function [37], and that obese, normoglycaemic Zucker rats develop vascular and neural dysfunction at 32–40 weeks of age [37, 38]. It has been reported that type 2 diabetes mellitus is one of the risk factors for cognitive impairment [39]. The pathogenesis of cognition dysfunction is only partially understood. Many studies suggest that changes in cerebral structure and function in diabetes are related to hyperglycaemia-induced end organ damage through the activation of the polyol pathway and protein kinase C, as well as accumulation of AGEs. Also, macrovascular disease and insulin resistance may play a role in pathogenesis of memory impairment in type 2 diabetes and obesity [40–42]. In our study, although at the end of experiment we observed working memory deficits in spontaneous alternation in rat model of obesity and type 2 diabetes, there were no significant associations between the spontaneous alterations in the Y-maze test and Glo1 activities in neuronal tissue of Goto-Kakizaki rats and Zucker rats. In the present study, before the appearance of functional impairments in the neuronal tissue of 24-week-old Goto-Kakizaki rats, reduced Glo1 activity was found in the brain, spinal cord and sciatic nerve. Other recent studies have characterised GLO1 expression in the peripheral nervous system in different murine strains and have shown that reduced GLO1 expression in a streptozotocin-induced hyperglycaemia model may lead to symptoms of diabetic neuropathy [43, 44]. However, in our study, GLO1 expression at the gene level was not significantly altered (data not shown), even though the activity was significantly reduced. Thus, it could be hypothesised that the changes in Glo1 activity are not due to any pro-inflammatory signalling at the expressional level, but rather reflect glycotoxicity-induced changes in glyoxalase system which might be mediated by altered cellular concentration of GSH [28]. It must be noted that the experimental Goto-Kakizaki model represents lipo- and glycotoxicity because the respective biochemical measures are elevated compared to the control animals, and changes in neuronal Glo1 activity correlate with elevated glucose and triglyceride concentrations. However, Glo1 activity is less sensitive to elevated lipid levels, as we did not find significant changes in Glo1 activity in Zucker fa/fa rats. This study provides evidence for disturbed neuronal Glo1 activity under conditions of hyperglycaemia in the presence of impaired endothelium-dependent relaxation and working memory.

In conclusion, this study provides evidence for disturbed neuronal Glo1 activity under conditions of hyperglycaemia in the presence of impaired endothelium-dependent relaxation and cognitive function. In addition, blood Glo1 activity could not be used as an early marker of the development of diabetic complications in experimental models of type 2 diabetes or metabolic syndrome.

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Conflict of interest The authors declare that there are no conflicts of interest.

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