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Regular moderate exercise reduces advanced glycation and ameliorates early diabetic nephropathy in obese Zucker rats

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Abstract

Advanced glycation end products (AGEs) play a key role in the pathogenesis of diabetes and its complications, including the diabetic nephropathy. The renoprotective effects of exercise are well known; however, the mechanisms remain elusive. Here we examined whether a regular moderate exercise in obese Zucker rats (OZR), a model of diabetes- and obesity-associated nephropathy, will affect the development of early renal injury in OZR possibly via alteration of AGEs formation. The OZR were left without exercise (sedentary) or subjected to 10 weeks intermittent treadmill running of moderate intensity. Compared with sedentary OZR, kidneys of running OZR had significantly less glomerular mesangial expansion and tubulointerstitial fibrosis. Running OZR had significantly lower plasma AGEs-associated fluorescence and N^{ϵ}-carboxymethyllysine. Correspondingly, renal AGEs and N^{ϵ}-carboxymethyllysine content were lower in running OZR. Systemically, exercise increased aerobic metabolism, as apparent from urinary metabolite profiling. No differences in plasma glucose, insulin, or lipid profile were found between the 2 groups. Apart from lower advanced oxidation protein products (a marker of myeloperoxidase activity), no other marker of inflammation was altered by exercise, either systemically or locally in kidneys. No indication of changed oxidative status was revealed between the groups. Exercise in OZR decreased advanced glycation. This might represent the early event of exercise-induced renoprotection in diabetic nephropathy in OZR. If confirmed in clinical studies, regular moderate exercise could represent an easy and effective nonpharmacologic approach to reduce advanced glycation.

1. Introduction

Advanced glycation end products (AGEs) are a heterogeneous group of compounds that accumulate in and contribute to the pathogenesis of a number of diseases including diabetes and its complications [1]. Advanced glycation end products are formed by nonenzymatic glycation, a reaction between sugars (eg, glucose or intermediates of glucose metabolism) and amino groups (eg, of peptides and proteins). Advanced glycation end products also arise during oxidative stress or inflammation. Because the main organ of AGEs disposal is the kidney, it is also one of the main targets of their deleterious effects. It is well established that AGEs induce pathologic changes in glomeruli of healthy or diabetic animals including mesangial expansion, fibrosis, and inflammation [1-6]. The effect of exercise on AGEs formation is unknown.

Prevalence of end-stage renal disease due to type 2 diabetes mellitus and obesity is increasing worldwide. Effective interventions preventing the development or halting the progression of renal disease are needed [7,8]. Regular moderate aerobic exercise was shown to normalize

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glucose metabolism, reduce inflammation, but also improve renal function in patients and experimental models of diabetes [9-14].

Here we have analyzed the effects of regular moderate exercise on advanced glycation (AGEs) and nephropathy in obese Zucker rats (OZR). Obese Zucker rat is a widely used animal model of type 2 diabetes mellitus– and obesity-associated nephropathy, characterized by increased AGEs formation [15-20]. Potential renoprotective effects were analyzed in the kidney glomeruli and the tubulointerstitium separately. We focused mainly on mesangial expansion, fibrosis, and inflammatory infiltration, processes that could be mediated by AGEs.

2. Methods

The study was approved by The State Veterinary and Food Control in Bratislava. Two animals per cage were held in a room with constant temperature and humidity and with 12-hour/12-hour light cycles and had ad libitum access to tap water and standard rat chow (SP1, Top Dovo, Czech Republic). Male Crl:ZUC-Lepr^{fa} OZR (Charles River, Wilmington, MA), aged 8 weeks (weighing 290 g), were randomized into 2 groups (each n = 8). Rats in the running group (RUN) were subjected to exercise on a 4-channel treadmill (Harvard Apparatus, Holliston, MA), whereas rats in the sedentary group (SED) were placed for the same period to turned-off treadmill. The exercise consisted of a 5-week accommodation phase with increasing exercise intensity (first, second, and third week: 15 m/min for 30, 45, and 60 minutes, respectively; fourth and fifth week: 20 m/min for 30 and 45 minutes, respectively), followed by a 5-week constant training period (20 m/min for 60 minutes). Before each training session (5 times a week, always between 8.00 and 9.00 AM), all running rats had a 5-minute warm-up phase with slowly increasing speed. Food consumption was measured on each day of exercise.

One day before killing (after the last training session), rats were placed into the metabolic cages for 24-hours to collect stool-free urine for biochemical analyses and ^{1}H nuclear magnetic resonance (NMR) spectroscopy.

Rats were killed by exsanguination via abdominal aorta (blood was used for biochemical analyses) under ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneal anaesthesia. Kidneys were weighed and immediately processed for further analyses. All samples, if not analyzed immediately, were stored at −80°C.

2.1. Biochemical analysis

Standard blood and urine parameters (Vitros 250 analyzer; J&J, Rochester, NY) and blood counts (Sysmex K-21 analyzer; Sysmex, Kobe, Japan) were measured. Plasma advanced oxidation protein products (AOPPs), markers of myeloperoxidase (MPO) activity, were determined spectrophotometrically (340 nm, MRX microplate reader; Dynatech Laboratories, Chantilly, VA) and expressed as micromoles per liter of chloramine-T equivalents [21]. The AGEs-specific fluorescence of the 50-fold diluted plasma samples was measured on a Fluorite 1000 spectrometer (λ_{ex}350 nm/λ_{em}450 nm, Dynatech Laboratories), as described previously [22]. Proteinuria was determined by the pyrogallol red method. Plasma tumor necrosis factor–α, interleukin-6 (both rat-specific enzyme-linked immunosorbent assay [ELISA]; Bender MedSystems, Vienna, Austria), C-reactive protein (rat-specific ELISA; Biovendor Laboratory Medicine, Modrice, Czech Republic), and insulin (rat-specific radioimmunoassay; LINCO Research, St Charles, MO) were analyzed using commercially available kits according to manufacturers' instructions. Total N^{ε}-carboxymethyllysine (CML) was determined in serum, urine, and homogenates of glomeruli and tubulointerstitium by a specific ELISA (Microcoat, Bernried, Germany) after release of protein/peptide bound CML by pretreatment of samples with Proteinase K (Roche, Mannheim, Germany) according to manufacturers' instructions [23]. The CML content in homogenates was normalized to protein content. Thiobarbituric acid–reacting substances, total antioxidant capacity, and ferric-reducing ability of plasma and renal cortex were determined as described previously [24-26]. At the end of the experiment, insulin sensitivity was evaluated using the homeostasis model assessment index, which was calculated as fasting plasma insulin level (in microunits per milliliter) × fasting plasma glucose level (in millimoles per liter)/22.5.

2.2. Renal morphology

Tissue for light microscopy and immunohistochemistry was fixed in methyl Carnoy solution and embedded in paraffin. Sections (4 μm) were stained with the periodic acid–Schiff reagent (PAS) and counterstained with hematoxylin. All histomorphologic analyses were done in a blinded manner.

2.2.1. PAS-stained sections

The number of glomeruli (percentage) exhibiting expansion of mesangial areas and the percentage of glomeruli exhibiting protein droplets were calculated in 100 consecutive glomeruli. Tubulointerstitial damage score (0-4) was determined as described [20,27]. Using the ImageJ software version 1.40g (http://rsb.info.nih.gov/ij/), mean glomerular tuft area was calculated in 90 consecutive glomeruli. The number of cortical glomeruli was assessed in 40 consecutive cortical fields.

2.3. Immunohistochemistry

Sections (4 μm) of methyl Carnoy–fixed renal tissues were processed as previously described [27]. For platelet-derived growth factor receptor–β (PDGFR-β) staining, antigen retrieval with the Trilogy system (Cell-Marque, Rocklin, CA) was performed. Primary antibodies included a murine monoclonal antibody (mAb) (clone 1A4) to α–

smooth muscle actin (α-SMA) (DAKO, Glostrup, Denmark); a murine mAb (clone ED-1) to CD68 present on monocytes, macrophages, and dendritic cells (Serotec, Oxford, United Kingdom); a murine mAb raised against human PDGFR-*β*, affinity purified polyclonal goat antibodies against human type I and type IV collagen (Southern Biotechnology, Birmingham, AL); and murine mAb (clone D33), plus appropriate negative controls as described previously [27]. All antibodies used are cross-reactive and specific for the appropriate rat proteins.

The evaluation of immunohistochemistry was performed similarly as described previously [20,27,28]. The number of infiltrating monocytes/macrophages (ED-1) in each tissue was counted separately in 25 to 30 glomeruli and in 16 interstitial view fields at 200× magnification. In the case of immunostaining for desmin, edges of glomerular tufts, reflecting podocyte de novo expression of desmin, were scored semiquantitatively as described previously [20]. Computer-based morphometry was performed using the ImageJ software version 1.40g (http://rsb.info.nih.gov/ij/). The percentage of positively stained area (collagen type I and IV, PDGFR-*β*) in each tissue was calculated separately for 25 to 30 glomeruli and in 16 interstitial fields representing almost the whole cortical area, with each field having an area of 0.37 mm^2. In all analyses, the investigator was unaware of the origin of the slides.

2.4. RNA extraction and analysis

Glomeruli and cortical tubulointerstitium were isolated by differential sieving as described previously [22,29]. RNA extraction and analyses were performed as previously described [22]. In brief, total RNA was isolated using the standard Trizol protocol (MRC, Cincinnati, OH); and the concentration and purity of RNA were measured spectrophotometrically using NanoDrop (NanoDrop Technologies, Wilmington, DE). For real-time polymerase chain reaction (PCR), Qiagen QuantiFast SYBR Green RT-PCR kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions. For the normalization of the data, the standard δ Ct method was used with peptidyl prolyl isomerase A (cyclophilin A) as the housekeeping gene. The expression of genes of interest was calculated as relative expression units in comparison with the SED group (arbitrarily set as 1). Primers used for real-time PCR are shown in Table 1.

2.5. Urinary metabolites (metabolomics)

^{1}H NMR spectroscopy was performed as follows: 520 μL of 24-hour urine sample was mixed with 220 μL of solution containing 0.2 mol/L phosphate buffer (to minimize pH variation), 1 mmol/L imidazole (pH reference for targeted metabolites profiling software), 0.3 mmol/L sodium 3-trimethylsilyl-(2,2,3,3-2H4)-1-propionate (internal standard), and 31.25 mmol/L sodium azide (to prevent bacterial growth) in D_2O (NMR locking substance), and transferred to a 4-mm NMR tube. ^{1}H NMR spectra were recorded on a Varian INOVA spectrometer (Varian, Palo Alto, CA) at a proton frequency of 600.133 MHz. For water signal suppression, a standard 1-dimensional nuclear Overhauser effect spectroscopy (1D NOESY) pulse sequence was used with 128 scans per sample collected into 64-K data points. The spectra were processed and analyzed using the Chenomx software (Chenomx, Edmonton, Alberta, Canada) with manual phase and baseline correction, reference deconvolution, autoreferencing to internal sodium 3-trimethylsilyl-(2,2,3,3-2H4)-1-propionate standard at 0.00 ppm, and pH adjustment according to imidazole. The metabolites were assigned according to published literature and targeted metabolite profiling using the Chenomx software and the internal Chenomx urinary metabolites database at pH 6 to 8.

2.6. Statistical analyses

All values are expressed as means ± SD. Comparison of the groups was performed using 2-tailed Mann-Whitney *U* test. *Statistical significance* was defined as $P < .05$.

3. Results

3.1. Body and kidney weight and standard biochemistry

There were no differences in body weight, kidney to body weight ratio, weight gain, or food consumption

Table 1
Primers used for real-time PCR to quantify gene expression

Gene	Forward primer	Reverse primer
PPIA	GTCTCTTTTCGCCGCTTGCT	TCTGCTGTCTTTGGAACTTTGTCTG
TGF-*β*	GAAGGACCTGGGTTGGAAGT	TACTGTGTGTCCAGGCTCCA
SOD1	GGTGGTCCACGAGAAACAAG	CAATCACACCACAAGCCAAG
SOD2	CCAAAGGAGAGTTGCTGGAG	GAACCTTGGACTCCCACAGA
CAT	ATCAGGGATGCCATGTTGTT	CCAGAAGTCCCAGACCATGT
MPO	GGAAGTGGCGAACTCAGTGG	AGGAGTTCCATAGGGCTGGC
PDGF-B	GCTCTTCCTGCCTCTCTGCT	CGGATGGAGTGGTCACTCAG
PDGFR-*β*	ACAACCGTACCTTGGGTGAC	CTTCACACGTACCAAGGTCAGT
Col1A	CAACCTCAAGAAGTCCCTGC	ACAAGCGTGCTGTAGGTGAA
RAGE	AGAACCAGGAGCCTGGGAA	GCTCAGCATAAGTGGCTCTCC

PPIA indicates peptidyl prolyl isomerase A; CAT, catalase; Col1A, collagen type 1A.

Table 2
Body weight, blood biochemistry, and histomorphometric data of RUN and SED OZR

	RUN (n = 8)	SED (n = 8)	*U* test (*P* value)
Body weight start (g)	283 ± 30	295 ± 33	NS
Body weight end (g)	483 ± 25	494 ± 29	NS
Food intake (g/d)	30 ± 2	31 ± 3	NS
Plasma albumin (g/L)	**35 ± 2**	**32 ± 2**	.009
Plasma cholesterol (mmol/L)	3.4 ± 0.3	3.3 ± 0.3	NS
Plasma triacylglycerols (mmol/L)	2.2 ± 0.5	2.3 ± 0.7	NS
Plasma glucose (mmol/L)	25 ± 2	25 ± 2	NS
Plasma insulin (ng/mL)	0.64 ± 0.21	0.80 ± 0.18	NS
Plasma creatinine (μmol/L)	35 ± 6	32 ± 5	NS
BUN (mmol/L)	5.3 ± 0.9	5.9 ± 0.6	NS
Creatinine clearance/1 g KW (mL/[min g])	0.51 ± 0.15	0.51 ± 0.15	NS
Proteinuria (mg/d)	**43 ± 15**	**24 ± 12**	.012
U-prot/U-crea (mg/μmol)	**0.83 ± 0.41**	**0.51 ± 0.37**	.027
Kidney to body weight ratio ($\times 10^3$)	4.7 ± 0.3	4.8 ± 0.2	NS
Glomerular tuft area (pixels $\times 10^3$)	9.5 ± 1.0	8.7 ± 1.1	NS
No. of glomeruli/view field	10 ± 1	11 ± 1	NS

Data are means ± SD. BUN indicates blood urea nitrogen; KW, kidney weight; U-prot/U-crea, urinary protein to creatinine ratio; NS, not significant. Significantly different parameters are shown in bold.

between the OZR subjected to training and those left sedentary (Table 2). Running OZR had slightly but significantly higher plasma albumin (+9%), although it was within the reference range. All other plasma parameters remained unaffected (creatinine, blood urea nitrogen, potassium, calcium, phosphorus, cholesterol, triacylglycerols, aspartate transaminase, alanine transaminase, alkaline phosphatase, γ-glutamyl transferase, and bilirubin; Table 2 or data not shown). Running OZR had significantly higher proteinuria (+77%) or urinary protein to creatinine ratio (+64%, Table 2). Exercise did not affect the other urinary parameters (24-hour excretion of creatinine, Na^+, K^+, P, Ca^{2+}, Mg^{2+}), even when normalized to urinary creatinine (data not shown). Blood count was within the reference range and did not differ between the groups (data not shown). No rats died or were excluded from the study.

3.2. Advanced glycation

Exercise resulted in significantly lower plasma AGEs-associated fluorescence (−14%, Fig. 1A). Correspondingly, plasma concentration of a chemically defined AGEs compound, CML, was lower in running rats (−27%, Fig. 1B). Urinary excretion of CML remained unaffected (RUN, 0.84 ± 0.60 μg/d vs SED, 0.90 ± 0.60 μg/d CML; not significant). The AGEs-associated fluorescence in renal cortex was significantly lower in the running OZR (−31%, Fig. 1D). In glomerular and tubulointerstitial homogenates of the exercising rats, CML content was reduced (−46% and −57%, respectively; Fig. 1E-F), albeit the latter not significantly (*P* = .085).

3.3. Mechanisms of reduced glycation

No significant differences in fasting plasma glucose or insulin concentrations were detected (Table 2). Homeostasis model assessment index of insulin resistance was similar in both groups (data not shown).

No difference was found in white blood cell counts or plasma C-reactive protein. Interleukin-6 and tumor necrosis factor–α were less than the detection limit in both groups (data not shown). Running OZR had lower AOPP, a marker of MPO reaction (−35%, Fig. 1C).

Markers of antioxidative capacity, total antioxidant capacity, and ferric-reducing ability of both plasma and renal cortex were similar in both groups (Supplementary Table 1). Thiobarbituric acid–reacting substances, a marker of oxidative damage to lipids, also did not differ between the groups, either in plasma or in kidney cortex (Supplementary Table 1). The cortical messenger RNA (mRNA) expression of antioxidant enzymes (superoxide dismutase [SOD] 1, and SOD2, and catalase; Supplementary Fig. 2I) was similar in running and sedentary OZR.

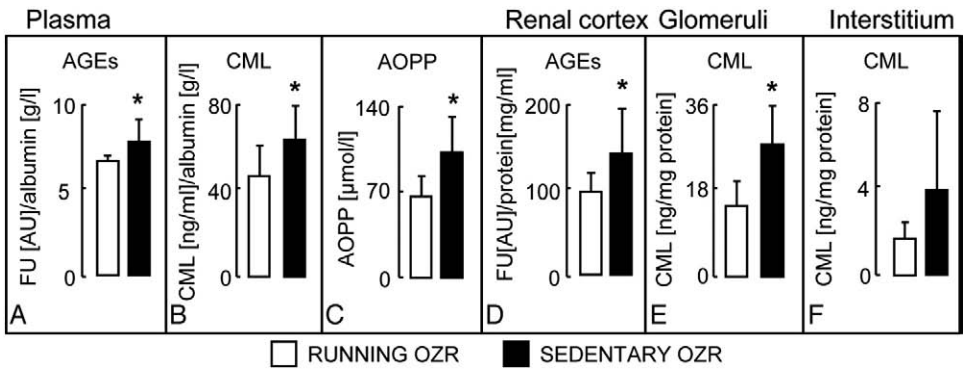

Fig. 1. Effects of regular moderate physical exercise on advanced glycation in OZR. Plasma AGEs-associated fluorescence (A), chemically defined AGEs CML (B), and AOPP (C), a marker of MPO reaction, were significantly lower in running OZR compared with sedentary OZR. In renal cortex, AGEs-associated fluorescence (D) and, in glomeruli, CML (E) were also significantly lower in running OZR. In the tubulointerstitium (F), the difference in CML content was not significant (*P* = .085) because of high variability. White bars represent running OZR (n = 8); and black bars, sedentary OZR (n = 8). All data are means ± SD; **P* < .05. AU indicates arbitrary units; FU, fluorescence units.

Table 3
Mean concentrations (in micromoles per liter) of some of the urinary metabolites analyzed by targeted metabolite profiling of ^{1}H NMR spectra in RUN and SED OZR

	RUN	SED	%	Metabolism
Succinate	**0.627 ± 0.274**	**0.450 ± 0.156***	**139**	Energy (TCA)
Oxaloacetate	*1.933 ± 0.736*	*1.601 ± 0.522*	*121*	Energy (TCA)
Citrate	9.553 ± 1.913	8.639 ± 1.770	111	Energy (TCA)
Acetate	*0.595 ± 0.470*	*0.357 ± 0.244*	*167*	Energy (*β*-oxidation)
Lactate	0.182 ± 0.096	0.173 ± 0.085	106	Energy (anaerobic)
1-Methylnicotinamide	**0.026 ± 0.011**	**0.035 ± 0.010***	**74**	Nicotinamide
Trigonelline	*0.126 ± 0.037*	*0.094 ± 0.030*	*133*	Nicotinate
Trimethylamine-N-oxide	*0.464 ± 0.136*	*0.364 ± 0.103*	*128*	Methane (gut microflora)

The concentrations are normalized to urinary creatinine (micromoles per micromole creatinine). The main difference found in running OZR was higher levels of metabolites involved in aerobic energy metabolism, whereas no difference in the metabolite of the anaerobic pathway lactate was observed. The "%" column shows the percentage value of the running group when the sedentary group was set as 100%. The "Metabolism" column shows the major metabolic pathway in which the metabolite is involved. Rows in bold indicate significantly different metabolite concentrations; rows in italics, those different by more than 20% but not significantly. TCA indicates tricarboxylic acid cycle.

* $P < .05$.

Targeted metabolite profiling of 23 major urinary metabolites from the ^{1}H NMR spectra revealed that, in the running OZR, intermediates mainly involved in aerobic energy metabolism were higher. Concentrations of urinary succinate were significantly higher, whereas those of 1-methylnicotinamide were significantly lower (Table 3).

3.4. Renal effects of exercise—glomeruli

The major pathologic finding in glomeruli of OZR was expansion of mesangial fields. At this stage, 9% to 34% of glomeruli exhibited mostly segmental mesangial expansion. Running significantly reduced the number of glomeruli with mesangial expansion (−31%, Fig. 2A-C). Platelet-derived growth factor receptor–*β*, which is expressed constitutively on mesangial cells [30], was significantly lower in running rats at the protein (−25%, Fig. 2F-H) and mRNA levels (−84%, Fig. 3). Messenger RNA of PDGF-B, the PDGFR-*β* ligand involved in mesangial proliferation [27,30], was reduced insignificantly (−60%, $P = .083$, Fig. 3). The marker for mesangial cell activation, α-SMA, was nearly completely absent in glomeruli; and no differences were observed between the groups (Fig. 2I-K).

A striking number of glomerular cells exhibited PAS-positive droplets localized to podocytes, an indicator of protein accumulation in proteinuria. The percentage of glomeruli with protein droplets was lower in the exercising rats (−39%, Fig. 2D-E). Both the number of cortical glomeruli and glomerular tuft area (a measure of glomerular hypertrophy) were unchanged by exercise (Table 2).

There was no difference in glomerular monocyte/macrophage counts (Supplementary Fig. 1A-C) or in glomerular MPO mRNA expression (Supplementary Fig. 1J) between the groups.

In some glomeruli, arteriolar hyalinosis was observed; and few areas of focal sclerosis were found in PAS staining. The basement membrane collagen type IV was not altered by running (Supplementary Fig. 1D-F), and there was no glomerular expression of scar collagen I either in running or in sedentary OZR (Supplementary Fig. 1G-I). No differences in mRNA expression of profibrotic genes (collagen type I, transforming growth factor-*β* [TGF-*β*]) and of the receptor for AGE (RAGE) were found in isolated glomeruli (Supplementary Fig. 1J).

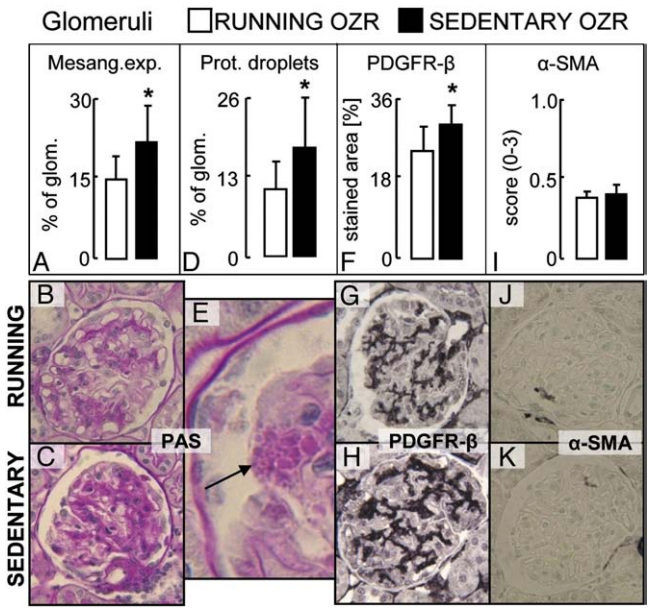

Fig. 2. Effects of regular moderate physical exercise on glomeruli of OZR. Running OZR exhibited lower number of glomeruli with mesangial expansion (A-C) and with protein droplets (D-E, arrow points to PAS-positive protein droplets presumably in a podocyte). Reduced mesangial expansion was confirmed by reduced glomerular area positive for mesangial marker PDGFR-*β* (F-H). No difference in activation of mesangial cells (glomerular α-SMA) was found (I-K). White bars represent running OZR (n = 8); and black bars, sedentary OZR (n = 8). All pictures (except E) are in original magnification ×630. A digitally magnified glomerular area with PAS-positive protein droplets (E). All data are means ± SD; **P* < .05. Col indicates collagen; glom, glomerulus; Mesang exp, mesangial expansion; Prot droplets, PAS-positive protein accumulation in podocytes.

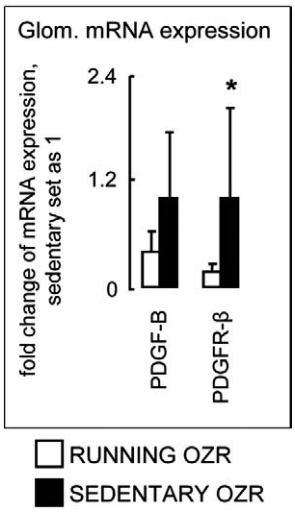

Fig. 3. Glomerular mRNA expression confirmed reduced PDGFR-β expression in running OZR. The PDGFR-β ligand PDGF-B was also reduced, but significance was not reached. White bars represent running OZR (n = 8); and black bars, sedentary OZR (n = 8). All data are means ± SD; *$P < .05$.

3.5. Renal effects of exercise—tubulointerstitium

In the tubulointerstitium, the main pathologic finding was mild fibrosis, mainly peritubular but also periglomerular. Tubules with thickened basement membrane, and a mild degree of atrophy and dilatation were also observed. Only very few inflammatory cell infiltrates (mostly perivascular) were noted. Tubulointerstitial damage score was insignificantly lower in the exercising group (−27%, Fig. 4A-C). Area positive for collagen type I was reduced significantly (−24%, Fig. 4D-F). The area stained for PDGFR-β, which is mainly expressed by fibroblasts [30], was reduced significantly in the exercising group at the protein level (−26%, Fig. 4G-I) but not at the mRNA level (−15%, not significant; Fig. 4J). In running OZR, the profibrotic cytokine TGF-β was significantly lower (−85%, Fig. 4J); and PDGF-B mRNA expression tended to be lower (−36%, not significant; Fig. 4J). No difference in collagen type IV, a constituent of basement membranes, was found (Supplementary Fig. 2A-C). At this stage of nephropathy, virtually no myofibroblasts were detected in tubulointerstitium of both groups with α-SMA staining (Supplementary Fig. 2G-H).

No difference was found between the groups regarding infiltration by monocytes/macrophages (Supplementary Fig. 2D-F) or MPO expression (Supplementary Fig. 2I).

4. Discussion

Here we have found that moderate regular exercise reduced the burden of AGEs in a rat model of type 2 diabetes mellitus– and obesity-associated nephropathy. Exercise affected glomeruli and tubulointerstitium differently, reducing mesangial expansion in the former and fibrosis in the latter.

A substantial body of evidence showed the renoprotective effects of moderate exercise in type 1 and type 2 diabetes mellitus patients as well as in animal models of diabetic nephropathy [9-14]. However, the early events leading to renoprotection were not yet identified. We initiated moderate

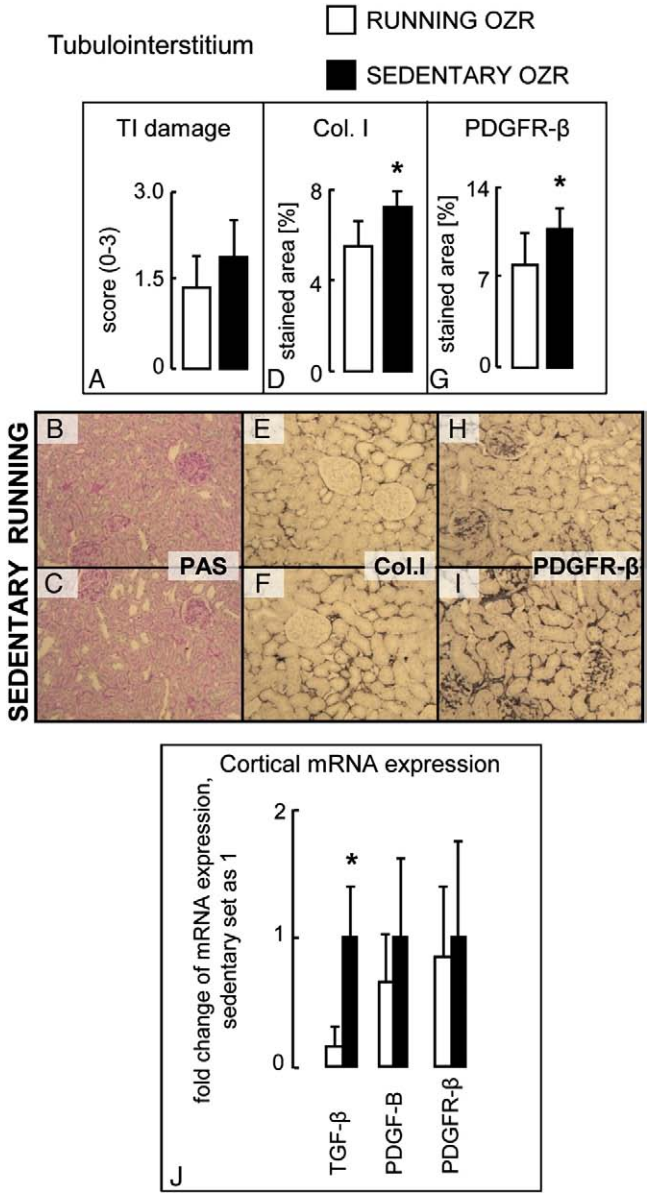

Fig. 4. Effects of regular moderate physical exercise on tubulointerstitium of OZR. Tubulointerstitial damage score was slightly and insignificantly lower in running OZR (A-C). Extracellular matrix deposition of collagen type I was significantly lower in running OZR (D-F); as was PDGFR-β, a marker of fibroblasts and pericytes (G-I). Tubulointerstitial mRNA expression showed significantly lower profibrotic TGF-β, whereas PDGF-B and PDGFR-β only tended to lower expression in running OZR. All pictures are in original magnification ×200. White bars represent running OZR (n = 8); and black bars, sedentary OZR (n = 8). All data are means ± SD; *$P < .05$. TI indicates tubulointerstitial.

physical exercise in young OZR (8 weeks of age) before any overt clinical signs of diabetic- or obesity-associated nephropathy were anticipated. Rats were allowed to eat ad libitum, but we found no differences in body weight or food consumption as was found previously [31]. This could be due to shorter and less intense exercise schedule compared with other experimental studies [11-14], or intermittent training (ie, not performed during weekends). Thus, naturally hyperphagic OZR could replenish the energy expenditure. In addition, we tried to mimic a standard exercise program, for example, warm-up period before each exercise, a run-in period before a steady full training phase, and an intermittent schedule (5 days a week).

Renal histology in sedentary OZR resembled the classic findings of diabetic nephropathy in this model, albeit still mildly accentuated, that is, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis [8,32]. In line with previous reports, exercise, albeit shorter and less intense, reduced mesangial expansion in OZR [11,13,14]. No activation of mesangial cells, assessed whether as α-SMA or as collagen type I expression in glomeruli, was observed at this early stage, as reported previously [20,28,33]. Platelet-derived growth factor receptor–β is constitutively expressed on mesangial cells and fibroblasts [30]. Exercise reduced PDGFR-β expression in glomeruli and the number of glomeruli exhibiting mesangial expansion on PAS sections, suggesting that exercise might decrease mesangial expansion before overt mesangial cell activation occurs. We found nearly no indication of activated (myo)fibroblasts in tubulointerstitium, as assessed by α-SMA staining. However, reduced tubulointerstitial PDGFR-β expression, collagen type I deposition, and TGF-β expression suggested that exercise ameliorated the early tubulointerstitial fibrotic changes in diabetic nephropathy in OZR.

Regular exercise decreased AGEs. Formation of AGEs is triggered by hyperglycemia, oxidative stress, and inflammation. We found no differences in plasma glucose, inflammatory markers or oxidative status, either systemically or locally. Renal excretion is the main pathway of AGE disposal, but comparable renal function and CML excretion in both groups exclude this mechanism. Thus, a different mechanism of exercise-induced reduction in advanced glycation might have been involved. Reactive intermediates of glycolytic (eg, glucose-6-phosphate; fructose-6-phosphate; fructose-1,6-diphosphate; glyceraldehyde-3-phosphate) and polyol (eg, fructose-3-phosphate, 3-deoxyglucosone) pathways are extremely potent glycating agents despite their much lower in vivo concentrations compared with glucose [1,34]. We presume that exercise-induced higher energy demands might decrease the pool of these reactive intermediates available for glycation, in particular in diabetic state. More effective utilization of these intermediates in metabolic cycles would explain the exercise-associated reduction in plasma and tissue AGEs. Although we did not study the intracellular levels of these intermediates, the profile of urinary metabolites clearly indicated higher aerobic energy metabolism in running OZR (Table 3). In addition, in the running OZR, reduced activation of phagocytes could also reduce glycation via MPO reaction (reduced AOPPs).

Reduction of AGEs by regular moderate exercise might have been a major mechanism of exercise-associated renoprotection in our OZR. In running OZR, we found reduced glomerular PDGF-B expression and mesangial expansion; and in tubulointerstitium, less fibrosis and TGF-β expression. The involvement of AGEs in the development of diabetic nephropathy, renal fibrosis, and vascular dysfunction is well described [1,8,32]. More specifically, AGEs induced the production of TGF-β and PDGF in mesangial cells, fibroblasts, and tubular cells [1,35]. Advanced glycation end products infusion or consumption of high-AGEs diet leads to renal damage in healthy animals, closely resembling diabetic nephropathy including mesangial expansion and fibrosis [2,5,6]. In the model of subtotal nephrectomy, high dietary AGEs intake promoted renal fibrosis [36]. Furthermore, anti-AGEs treatments in experimental diabetic models ameliorated the disease course [3,4].

Exercise did not influence the systemic or local inflammation markers. This is in agreement with the recent finding that exercise in hypertensive uninephrectomized diabetic rats did not alter the renal infiltration of ED-1–positive cells [14]. However, running OZR had lower circulating AOPP levels, a marker of oxidative damage to proteins induced via MPO reaction from activated monocytes/macrophages [21]. This suggests that exercise might modulate monocyte/macrophage activation rather than their numbers. Lower AOPP levels might be renoprotective per se because AOPPs directly aggravated renal fibrosis and increased cortical TGF-β expression in rat remnant or diabetic kidneys [37,38].

Pharmacologic interventions [15,16], physical training [12,13], and food restriction [39] were reported to be associated with a decline in proteinuria or at least with its stabilization, if intervention was initiated later on [39]. The obvious difference to these studies was higher proteinuria in our running OZR. This is most likely due to the well-known postexercise proteinuria because, in our study, OZR were placed in metabolic cages for urine collection directly after the last training session [40,41]. The lower number of glomeruli exhibiting protein droplets in running OZR is reassuring. However, the timing of urine collection right after the last exercise represents a drawback of this study design.

In conclusion, regular moderate exercise decreased advanced glycation in OZR independently of alimentary AGEs intake, glycemia, insulin sensitivity, or oxidative stress. The early exercise-associated renoprotection in OZR involved reduced glomerular mesangial expansion and reduced tubulointerstitial fibrosis. These effects could be attributed, at least in a part, to reduced AGEs. We show here—to our knowledge, for the first time—that regular

moderate exercise might be an easy and effective strategy to reduce advanced glycation in diabetes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.05.025.

References

[1] Bohlender JM, Franke S, Stein G, Wolf G. Advanced glycation end products and the kidney. Am J Physiol Renal Physiol 2005;289: F645-59.
[2] Cai W, He JC, Zhu L, et al. Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. Am J Pathol 2008;173:327-36.
[3] Forbes JM, Thallas V, Thomas MC, et al. The breakdown of preexisting advanced glycation end products is associated with reduced renal fibrosis in experimental diabetes. FASEB J 2003;17:1762-4.
[4] Sugimoto H, Grahovac G, Zeisberg M, Kalluri R. Renal fibrosis and glomerulosclerosis in a new mouse model of diabetic nephropathy and its regression by bone morphogenic protein–7 and advanced glycation end product inhibitors. Diabetes 2007;56:1825-33.
[5] Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, Steffes M. Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. Proc Natl Acad Sci U S A 1994;91: 11704-8.
[6] Yang CW, Vlassara H, Peten EP, He CJ, Striker GE, Striker LJ. Advanced glycation end products up-regulate gene expression found in diabetic glomerular disease. Proc Natl Acad Sci U S A 1994;91: 9436-40.
[7] Locatelli F, Pozzoni P, Del Vecchio L. Renal manifestations in the metabolic syndrome. J Am Soc Nephrol 2006;17:S81-5.
[8] Jefferson JA, Shankland SJ, Pichler RH. Proteinuria in diabetic kidney disease: a mechanistic viewpoint. Kidney Int 2008;74:22-36.
[9] Johansen KL. Exercise in the end-stage renal disease population. J Am Soc Nephrol 2007;18:1845-54.
[10] Evans N, Forsyth E. End-stage renal disease in people with type 2 diabetes: systemic manifestations and exercise implications. Phys Ther 2004;84:454-63.
[11] Albright AL, Mahan JD, Ward KM, Sherman WM, Roehrig KL, Kirby TE. Diabetic nephropathy in an aerobically trained rat model of diabetes. Med Sci Sports Exerc 1995;27:1270-7.
[12] Chiasera JM, Ward-Cook KM, McCune SA, Wardlaw GM. Effect of aerobic training on diabetic nephropathy in a rat model of type 2 diabetes mellitus. Ann Clin Lab Sci 2000;30:346-53.
[13] Ward KM, Mahan JD, Sherman WM. Aerobic training and diabetic nephropathy in the obese Zucker rat. Ann Clin Lab Sci 1994;24: 266-77.
[14] Tufescu A, Kanazawa M, Ishida A, et al. Combination of exercise and losartan enhances renoprotective and peripheral effects in spontaneously type 2 diabetes mellitus rats with nephropathy. J Hypertens 2008;26:312-21.
[15] Blanco S, Bonet J, Lopez D, Casas I, Romero R. ACE inhibitors improve nephrin expression in Zucker rats with glomerulosclerosis. Kidney Int Suppl 2005:S10-4.
[16] Blanco S, Penin R, Casas I, Lopez D, Romero R. Effects of antihypertensive drugs in experimental type 2 diabetes-related nephropathy. Kidney Int Suppl 2002:S27-31.
[17] Sebekova K, Eifert T, Klassen A, Heidland A, Amann K. Renal effects of S18886 (Terutroban), a TP receptor antagonist, in an experimental model of type 2 diabetes. Diabetes 2007;56:968-74.
[18] Sebekova K, Lill M, Boor P, Heidland A, Amann K. Functional and partial morphological regression of established renal injury in the obese Zucker rat by blockade of the renin-angiotensin system. Am J Nephrol 2009;29:164-70.
[19] Kasiske BL, O'Donnell MP, Keane WF. The Zucker rat model of obesity, insulin resistance, hyperlipidemia, and renal injury. Hypertension 1992;19:I110-5.
[20] Coimbra TM, Janssen U, Grone HJ, et al. Early events leading to renal injury in obese Zucker (fatty) rats with type II diabetes. Kidney Int 2000;57:167-82.
[21] Witko-Sarsat V, Friedlander M, Nguyen Khoa T, et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. J Immunol 1998;161: 2524-32.
[22] Boor P, Casper S, Celec P, et al. Renal, vascular and cardiac fibrosis in rats exposed to passive smoking and industrial dust fiber amosite. J Cel Mol Med 2009 [in press].
[23] Boehm BO, Schilling S, Rosinger S, et al. Elevated serum levels of *N* (epsilon)-carboxymethyl-lysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema. Diabetologia 2004;47:1376-9.
[24] Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol 1998;108:101-6.
[25] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239:70-6.
[26] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277-85.
[27] Boor P, Konieczny A, Villa L, et al. PDGF-D inhibition by CR002 ameliorates tubulointerstitial fibrosis following experimental glomerulonephritis. Nephrol Dial Transplant 2007;22:1323-31.
[28] Lavaud S, Michel O, Sassy-Prigent C, et al. Early influx of glomerular macrophages precedes glomerulosclerosis in the obese Zucker rat model. J Am Soc Nephrol 1996;7:2604-15.
[29] Bokemeyer D, Panek D, Kramer HJ, et al. In vivo identification of the mitogen-activated protein kinase cascade as a central pathogenic pathway in experimental mesangioproliferative glomerulonephritis. J Am Soc Nephrol 2002;13:1473-80.
[30] Floege J, Eitner F, Alpers CE. A new look at platelet-derived growth factor in renal disease. J Am Soc Nephrol 2008;19:12-23.
[31] Lash JM, Sherman WM, Betts JJ, Hamlin RL. Training-induced vascular and metabolic adaptations in normo(11 week)- and hyper(18 week)-glycemic obese Zucker rats. Int J Obes 1989;13:777-89.
[32] Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab 2008;4:444-52.
[33] Phillips AO, Baboolal K, Riley S, et al. Association of prolonged hyperglycemia with glomerular hypertrophy and renal basement membrane thickening in the Goto Kakizaki model of non–insulin-dependent diabetes mellitus. Am J Kidney Dis 2001; 37:400-10.

[34] Hamada Y, Araki N, Koh N, Nakamura J, Horiuchi S, Hotta N. Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. Biochem Biophys Res Commun 1996;228:539-43.

[35] Xiang G, Schinzel R, Simm A, et al. Advanced glycation end products (AGEs)–induced expression of TGF-beta 1 is suppressed by a protease in the tubule cell line LLC-PK1. Nephrol Dial Transplant 2001;16:1562-9.

[36] Feng JX, Hou FF, Liang M, et al. Restricted intake of dietary advanced glycation end products retards renal progression in the remnant kidney model. Kidney Int 2007;71:901-11.

[37] Li HY, Hou FF, Zhang X, et al. Advanced oxidation protein products accelerate renal fibrosis in a remnant kidney model. J Am Soc Nephrol 2007;18:528-38.

[38] kShi XY, Hou FF, Niu HX, et al. Advanced oxidation protein products promote inflammation in diabetic kidney through activation of renal nicotinamide adenine dinucleotide phosphate oxidase. Endocrinology 2008;149:1829-39.

[39] Maddox DA, Alavi FK, Santella RN, Zawada Jr ET. Prevention of obesity-linked renal disease: age-dependent effects of dietary food restriction. Kidney Int 2002;62:208-19.

[40] Poortmans JR. Evidence of increased glomerular permeability to proteins during exercise in healthy men. Contrib Nephrol 1988;68: 136-40.

[41] Poortmans JR, Vanderstraeten J. Kidney function during exercise in healthy and diseased humans. An update. Sports Med 1994;18: 419-37.