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MODULATION OF SYMPATHOADRENERGIC CONTRACTIONS BY PERIVASCULAR ADIPOSE TISSUE IN MESENTERIC ARTERIES OF RATS WITH DIFFERENT LEVEL OF BODY ADIPOSITY

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Obesity is associated with increased sympathetic nervous system activation, possibly contributing to higher cardiovascular risk. The aim of this study was to assess the relationship between body adiposity and sympathoadrenergic contractions in rat isolated mesenteric arteries, and the modulatory effect of mesenteric perivascular adipose tissue (PVAT). Experiments were performed on male 38-week-old Wistar, Zucker lean (ZL) and Zucker diabetic fatty (ZDF) rats. Paired rings of isolated rat superior mesenteric arteries with or without PVAT were prepared and connected to a force-displacement transducer for the recording of isometric tension. Contractile responses were elicited by increasing doses of exogenous noradrenaline and by endogenous noradrenaline released during electrical stimulation of perivascular adrenergic nerves. In ZDF rats, mesenteric PVAT had marked anticontractile effect leading to significant reduction in adrenergic contractions of their superior mesenteric arteries; however, in arterial preparations without PVAT, obese rats showed significantly increased sensitivity in their contractile responses to adrenergic stimulation when compared to other rat groups. In Wistar rats, ranging in the level of body adiposity between ZL and ZDF rats, neurogenic contractions in arterial preparations with preserved PVAT were higher compared to those without PVAT. No vasomodulatory effect of PVAT was detected in mesenteric arteries from ZL rats. The results of this study indicate that the modulatory effect of mesenteric PVAT on arterial adrenergic contractions did not change in proportion with increasing adiposity; however, it could be influenced by the rat strain-specific distribution of sympathetic nerves between PVAT and the proper mesenteric arterial wall. In ZDF rats, characterized by higher vascular sympathetic tone, the mesenteric arteries might be specifically regulated by the anticontractile effect of PVAT, leading to higher mesenteric blood flow. This could be associated with hyperphagia and increased nutrient-induced mesenteric vasodilatation in this rat strain.

Key words: *perivascular adipose tissue, mesenteric artery, adrenergic contraction, noradrenaline, Zucker diabetic fatty rat, sympathetic nervous system, lipid peroxidation, oxidative stress*

INTRODUCTION

The prevalence of obesity is progressively increasing in the developing world, due to altered patterns of nutrition and reduced energy expenditure, as well as environmentally induced epigenetic changes in human genome (1). In individual patients, high body mass index often coexists with several cardiometabolic diseases like hypertension, insulin resistance, hyperinsulinemia, and hyperlipidemia, this clustering of adverse health factors being designated the metabolic syndrome. Furthermore, obesity is often associated with hyperfunction of sympathetic nervous system, which could represent one of the pathophysiological mechanisms by which it might be linked to cardiovascular impairment and hypertension. Evidences for increased sympathetic activity in overweight and obese subjects were yielded using diverse methods like determination of plasma and urine catecholamines, systemic and regional noradrenaline turnover, and direct sympathetic nerve recording, or microneurography (2, 3).

In our previous studies we have demonstrated that vascular sympathetic activity might be directly influenced by perivascular adipose tissue (PVAT) which surrounds and is closely adjacent to the vessel surface (4). It was found that healthy perivascular adipocytes produce transferable factor(s) termed adipocyte-derived relaxing factor(s) (ADRF) which hyperpolarize vascular smooth muscle cells by activating different types of potassium channels on their membrane, leading to inhibition of vascular (sympathetic) contraction (5, 6). A number of PVAT-derived "anticontractile" molecules have been identified so far; besides adipokines (*e.g.*, adiponectin) these include also cytokines/chemokines, gaseous molecules (nitric oxide, hydrogen sulphide), angiotensin 1-7, methyl palmitate, and reactive oxygen species (hydrogen peroxide) (7-10). However, there are many evidences that in pathological conditions such as obesity and related cardiometabolic diseases,

PVAT could contribute to the impairment of vessel function by releasing of substances that support inflammation, smooth muscle cell migration, and atherogenesis (11-13). It was shown that in obese patients with metabolic syndrome the total PVAT mass around small arteries was increased while its anticontractile effect was completely lost, and markers of hypoxia and inflammation were detected (14). Similar results were found also in animal genetic and diet-induced models of obesity (15, 16).

The aim of this study was to examine the effect of PVAT on arterial sympathoadrenergic contractions in rats with different level of body adiposity. Investigating the relationship between the different amount of body fat and vasomodulatory effect of PVAT with direct impact on arterial sympathetic outflow might clarify the frequent association and link between metabolic and cardiovascular diseases.

MATERIALS AND METHODS

Experimental animals

All procedures and experimental protocols were performed in accordance with institutional guidelines and were approved by the State Veterinary and Food Administration of the Slovak Republic and by the Ethical Committee of the Institute of Pharmacology and Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Directive 2010/63/EU of the European Parliament.

Male Wistar rats (n = 8), Zucker lean (ZL; n = 8) rats and Zucker diabetic fatty (ZDF; n = 9) rats were used in the study. All animals were housed under a 12 h light-12 h dark cycle, at a constant temperature (20 – 22°C) and with free access to drinking water and food (Purina Rodent LabDiet 5008, IPS Product Supplies, UK). The content of fat in the diet was 6.5% (16.7% of calories).

Rats were sacrificed at the age of 38 weeks in non-fasting conditions under sevoflurane anesthesia. Trunk blood was collected in the heparinized tubes and immediately centrifuged (850 × g, 10 min, 4°C), and blood plasma was stored at –80°C till the analysis. Plasma glucose concentration was determined by the glucose oxidase method by using the analyser RX Monza, kit Randox (UK). Plasma insulin was measured using the ELISA procedure with the Rat/Mouse Insulin ELISA Kit (EZRMI-13K, Merck-Millipore, Germany). In each rat, retroperitoneal and epididymal fat depots were weighed, and the weights of heart, liver and left kidney were normalized to tibia length. Superior mesenteric artery was removed from each rat and prepared for isometric tension recording.

Functional studies on isolated mesenteric arteries

Superior mesenteric arteries were isolated from individual rats and quickly transferred to cold Krebs solution, and dissected into paired rings (2.8 – 3.2 mm in length) - one with PVAT preserved and other with PVAT removed. In the case of rings with PVAT removed (PVAT–), the perivascular fat was removed from arterial surface under a microscope with fine scissors, being careful not to damage the adventitia. In the case of rings with PVAT preserved (PVAT+), a continuous layer of perivascular fat (1 to 1.2 mm in width) was left around the arterial ring. Special caution was given not to damage the endothelial layer during preparation of each arterial ring. The rings were not tested for intactness of endothelium before the measurement protocol.

Each arterial ring was set up for isometric tension recording using a force-displacement transducer Sanborn FT 10 (Sanborn, Baltimore, USA), and suspended in 20 ml organ baths filled with oxygenated (95% O_2 + 5% CO_2) modified Krebs solution maintained at 37°C. The Krebs solution was prepared in the following composition (in mmol/l): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, $CaNa_2$ EDTA 0.03. The preparations were equilibrated under a resting tension of 10 mN for 60 – 90 min, and the Krebs solution was changed every 15 min.

Adrenergic contractions were determined in mesenteric arteries as the responses to increasing concentrations of exogenous noradrenaline applied into the bath in a cumulative manner, or as the neurogenic contractions elicited by transmural electrical stimulation of periarterial sympathetic nerves. The arterial rings were electrically stimulated by two parallel platinum plate electrodes placed on either side of the preparation and connected to an electrostimulator ST-3 (Medicor, Budapest, Hungary). Frequency-response curves to electrical stimuli were obtained using square pulses of 0.5 ms in duration, at supramaximal voltage (> 30 V), applied at 1 – 32 Hz, for a period of 20 s. In our previous observations we found that the contractions of rat mesenteric arteries elicited by transmural electrical stimulation, using the described parameters of stimulation, are blocked by phentolamine or tetrodotoxin, indicating that they are induced mainly by nerve-released noradrenaline (17).

Adrenergic contractile responses were additionally assessed in relation to the values of plasma glucose concentration in ZDF rats. Due to the heterogeneity of these rats in their level of glycaemia, this experimental group was further arbitrarily divided according to the levels of glucose and levels of insulin: one part of ZDF rat group was in the compensated phase of insulin resistance, having relatively lower values of plasma glucose (less than 16 mmol/l) and high levels of insulin; the other part had high levels of glucose (greater than 16 mmol/l) and lower insulinaemia. The comparison was made between these two subgroups of ZDF rats in their adrenergic contractile responses, in both PVAT+ and PVAT– arterial rings.

Arterial isometric contractile responses to particular stimuli were expressed as the active wall tension in mN and normalized to the length of the respective ring preparation (mm). The maximum contraction (Emax) and the negative log10 concentration required to achieve the half-maximum contraction (pEC_{50}) were determined for each concentration-response curve to express the sensitivity to noradrenaline. Area under curves (AUC, in arbitrary units) were calculated from individual concentration- or frequency-response curves in each of the experimental groups.

Tissue concentration of thiobarbituric acid-reactive substances and conjugated dienes

Thiobarbituric acid-reactive substances (TBARS) and conjugated dienes (CD), markers of lipid peroxidation, were measured in 10% tissue homogenates of the left ventricle, kidney and liver as described by Hu *et al.* (18), with some modifications. Adjustment of TBARS measurement is described in our previous work (19). To determine CD, 0.5 ml of chloroform and 1 ml of methanol was added into 0.4 ml of tissue homogenate, and vortexed for 1 min. Subsequently, 0.5 ml of chloroform was added, vortexed for 30 s, and finally 0.5 ml of redistilled water was added and vortexed for another 30 s. The tubes were sealed with parafilm and centrifuged at 424 g and 4°C (Eppendorf 5430R, Germany). 0.5 ml of the lower chloroform layer was pipetted to the tube and evaporated in warm water (45 – 55°C) under N_2 (99.5%) atmosphere for approximately 3 – 5 minutes. Next, 2 ml of cyclohexane was added, vortexed and let stand in the dark for 10 min. The absorbance of the samples was measured at 233 nm. An extinction coefficient of 26000 mol^{-1} 1 cm^{-1} was used for

calculation of results. The results were expressed in nmol of TBARS/CD per gram of tissue.

Data analysis

The results are presented as means ± standard errors of the means (SEM). GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) was used for the statistical analyses. Statistical evaluation was carried out by using one-way analysis of variance (ANOVA); the normality of TBARS and CD data were tested with the Shapiro-Wilk test. The concentration-response curves and the frequency-response curves were analysed by two-way ANOVA. The results were considered to be significant when $p < 0.05$.

RESULTS

Table 1 shows that ZDF rats had markedly higher whole body weight, retroperitoneal fat weight, relative liver weight, and plasma glucose and insulin concentrations, when compared to Wistar and ZL rats. Epididymal fat weight was significantly higher in ZDF than in ZL rats, but there was no difference in this parameter between Wistar and ZDF rats. ZL rats had significantly smaller body weight, retroperitoneal and epididymal fat weight, indicating the lowest level of body adiposity, but they had higher glycaemia comparing to Wistar rats; these two groups had similar values of relative liver weight.

ZDF rats had the highest values of relative heart and kidney weight when compared to Wistar and ZL rats. Wistar rats had significantly smaller relative heart weight but higher kidney weight than ZL rats (*Table 1*).

No significant differences between particular experimental groups were found in conjugated diene concentrations measured in the tissue samples of liver and left heart ventricle; however, in kidney tissue, their concentration was significantly decreased in ZDF comparing to Wistar rats. Concentration of thiobarbituric acid-reactive substances was increased in the liver of both ZL and ZDF rats when compared to Wistar rats (*Table 2*).

In isolated superior mesenteric arteries with PVAT on their surface (PVAT+), the neurogenic contractile responses were not

Table 1. Selected biometric and metabolic characteristics of 38-week-old Wistar rats, Zucker lean (ZL) rats and Zucker diabetic fatty (ZDF) rats.

	Wistar rats (n = 8)	**ZL rats** (n = 8)	**ZDF rats** (n = 9)	
Body weight (g)	490.4 ± 27.3	419.2 ± 12.0*	572.2 ± 21.8	* +++
Retroperitoneal fat weight (mg)	4461.9 ± 740.9	1754.4 ± 112.9***	18537.2 ± 1376.7	*** +++
Epididymal fat weight (mg)	5815.0 ± 1028.5	1534.4 ± 94.4***	6188.9 ± 284.4	+++
Tibia length (mm)	38.9 ± 0.9	40.6 ± 0.5	38.7 ± 0.2	++
Liver weight/tibia length (mg/mm)	323.6 ± 19.9	320.8 ± 13.6	757.6 ± 49.7	*** +++
Heart weight/tibia length (mg/mm)	30.1 ± 1.3	33.4 ± 0.7 *	39.4 ± 2.1	*** +
Kidney weight/tibia length (mg/mm)	34.9 ± 1.5	30.8 ± 0.9 *	43.1 ± 1.3	*** +++
Glucose (mmol/l)	6.1 ± 0.1	6.8 ± 0.2 *	19.8 ± 2.3	*** +++
Insulin (mmol/l)	4.2 ± 0.7	3.3 ± 0.1	19.0 ± 2.9	*** +++

Values represent means ± SEM; *$p < 0.05$, ***$p < 0.001$ versus Wistar rats; +$p < 0.05$, ++$p < 0.01$, +++$p < 0.001$ versus ZL rats.

Table 2. Concentration of thiobarbituric acid-reactive substances (TBARS) and conjugated dienes (CD) in selected tissues from Wistar rats, Zucker lean (ZL) rats and Zucker diabetic fatty (ZDF) rats.

		Wistar rats (n = 8)	**ZL rats** (n = 8)	**ZDF rats** (n = 6)
CD (nmol/g tissue)	**Left heart ventricle**	865.4 ± 118.9	968.1 ± 44.5	931.5 ± 50.1
	Left kidney	1181.0 ± 60.4	1095.8 ± 70.7	887.4 ± 82.1*
	Liver	1238.3 ± 34.6	1191.8 ± 25.4	1321.9 ± 59.7
TBARS (nmol/g tissue)	**Left heart ventricle**	14.9 ± 1.6	12.5 ± 1.4	13.3 ± 1.7
	Left kidney	43.1 ± 2.9	53.3 ± 1.9	56.9 ± 5.0
	Liver	24.4 ± 1.8	36.1 ± 2.7*	49.5 ± 4.7***

Values represent means ± SEM; *$p < 0.05$, ***$p < 0.001$ versus Wistar rats.

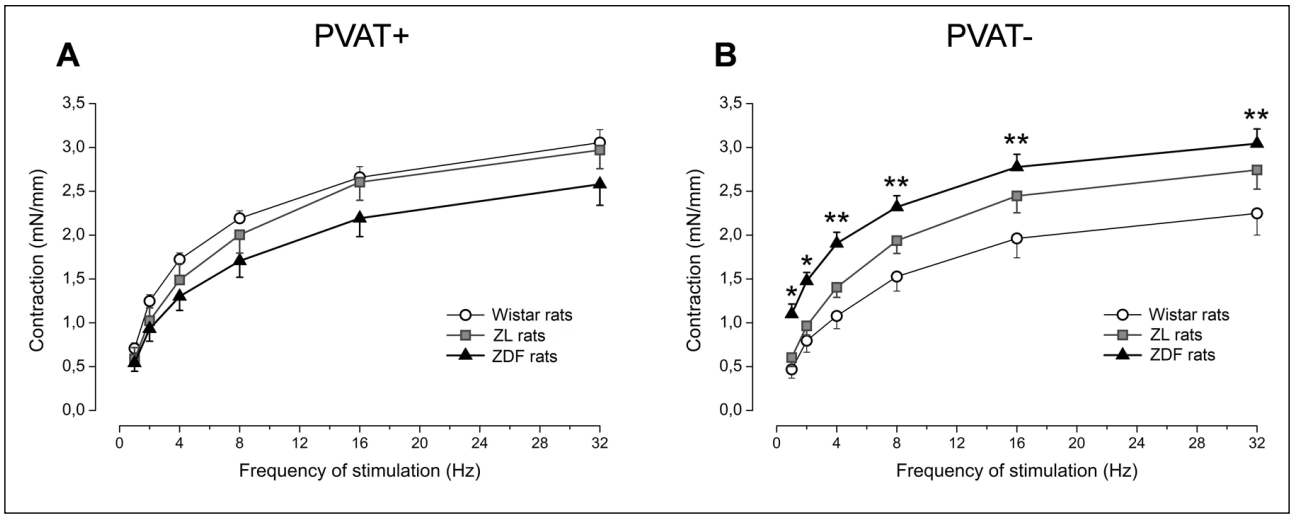

Fig. 1. Frequency-dependent neurogenic contractile responses to transmural electrical stimulation in superior mesenteric arteries from Wistar rats, Zucker lean (ZL) rats and Zucker diabetic fatty (ZDF) rats: arterial preparations (A) with PVAT and (B) without PVAT on their surface. Values represent means ± SEM; n = 8 – 9; *p < 0.05, **p < 0.01 versus Wistar rats.

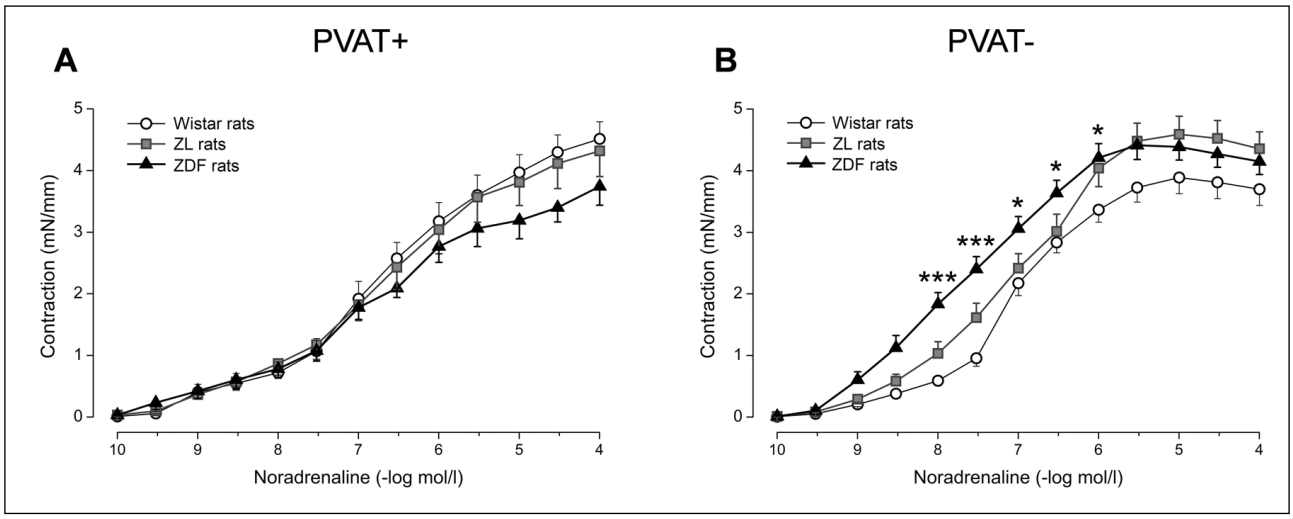

Fig. 2. Concentration-dependent contractile responses to exogenous noradrenaline in superior mesenteric arteries from Wistar rats, Zucker lean (ZL) rats and Zucker diabetic fatty (ZDF) rats: arterial preparations (A) with PVAT and (B) without PVAT on their surface. Values represent means ± SEM; n = 8 – 9; *p < 0.05, ***p < 0.001 versus Wistar rats.

Table 3. Characteristics of dose-response curves to exogenous noradrenaline in mesenteric arteries with perivascular adipose tissue removed (PVAT–) and intact (PVAT+), obtained from Wistar rats, Zucker lean (ZL) rats and Zucker diabetic fatty (ZDF) rats.

	Wistar rats (n = 8)		**ZL rats** (n = 8)		**ZDF rats** (n = 9)	
Noradrenaline	**PVAT–**	**PVAT+**	**PVAT–**	**PVAT+**	**PVAT–**	**PVAT+**
pEC_{50} (mol/l)	7.06 ± 0.09	6.61 ± 0.26	7.11 ± 0.13	6.70 ± 0.18	7.86 ± 0.12 *** +++	6.59 ± 0.20 ###
Emax (mN/mm)	3.90 ± 0.26	4.51 ± 0.28	4.61 ± 0.29	4.32 ± 0.42	4.42 ± 0.23	3.74 ± 0.31

pEC_{50}, negative logarithm of the half maximal effective noradrenaline concentration; Emax, maximum response to noradrenaline. Values represent means ± SEM; ***p < 0.001 versus PVAT– in Wistar rats; +++p < 0.001 versus PVAT– in ZL rats; ###p < 0.001 PVAT+ versus PVAT– within the ZDF rat group.

significantly different between the experimental groups (*Fig. 1A*). Likewise, in PVAT+ arterial preparations, the particular dose-dependent contractile responses to exogenous noradrenaline (*Fig. 2A*) together with Emax and pEC_{50} values for noradrenaline dose-response curves (*Table 3*) were similar between the rat groups.

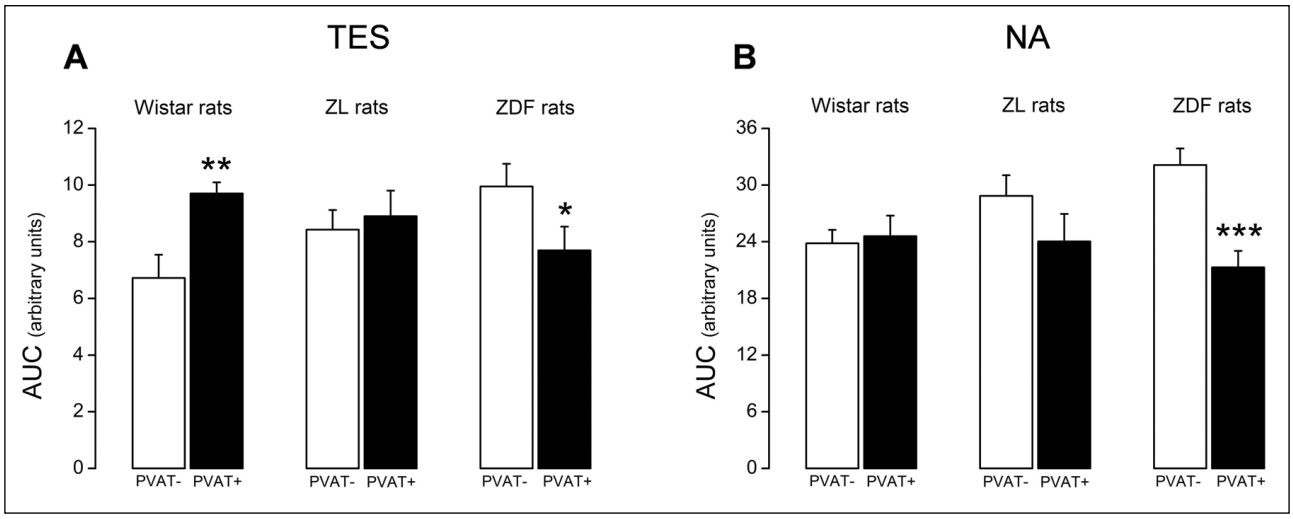

Fig. 3. (A) Frequency-dependent contractions to transmural electrical stimulation (TES) and (B) concentration-dependent contractions to exogenous noradrenaline (NA) expressed as AUC (area under curve) values in superior mesenteric arteries from Wistar rats, Zucker lean (ZL) and Zucker diabetic fatty (ZDF) rats: comparison between preparations with PVAT(+) and without PVAT(–) on their surface. Values represent means ± SEM; n = 8 – 9; *p < 0.05, **p < 0.01, ***p < 0.001 PVAT+ versus PVAT– (in the respective rat group).

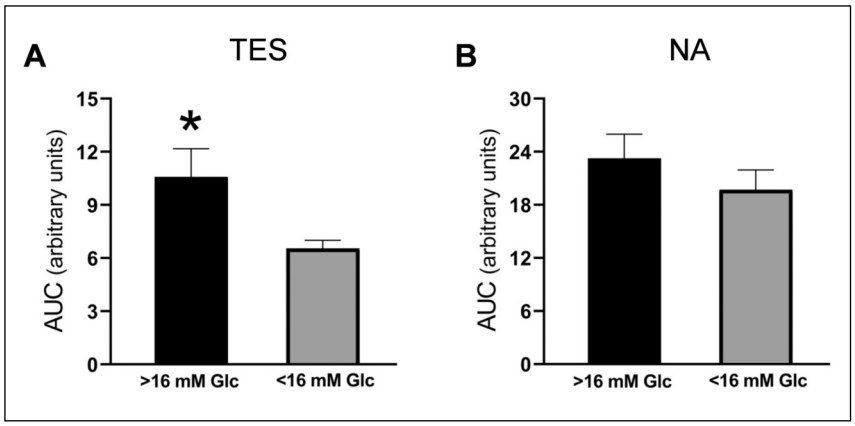

Fig. 4. (A) Frequency-dependent contractions to transmural electrical stimulation (TES) and (B) concentration-dependent contractions to exogenous noradrenaline (NA) expressed as AUC (area under curve) values in PVAT+ preparations of superior mesenteric arteries from Zucker diabetic fatty (ZDF) rats: comparison between ZDF rats with plasma glucose concentration greater than 16 mmol/l (> 16 mM Glc) and ZDF rats with plasma glucose concentration less than 16 mmol/l (< 16 mM Glc). Values represent means ± SEM; n = 4 (for < 16 mmol/l Glc) and n = 5 (for > 16 mmol/l Glc); *p < 0.05 versus < 16 mmol/l Glc.

In mesenteric arterial preparations without PVAT on their surface (PVAT–), ZDF rats had significantly increased contractions to electrical nerve stimulation and to exogenous noradrenaline comparing to Wistar rats (*Fig. 1B* and *2B*). The values of pEC_{50} for noradrenaline dose-response curves were significantly increased in ZDF rats in comparison with ZL and Wistar rat groups (*Table 3*), indicating higher arterial sensitivity to adrenergic stimulation in ZDF rats.

As shown in *Fig. 3* presenting AUC values for frequency- and dose-response curves, mesenteric arteries from Wistar rats had significantly higher neurogenic contractile responses in PVAT+ than in PVAT– preparations (*Fig. 3A*); however, PVAT had no effect on their exogenous noradrenaline-induced contractions (*Fig. 3B*). In ZL rats, no modulatory effects of PVAT were detected in contractile responses to electrical stimulation of sympathetic nerves and to exogenous noradrenaline (*Fig. 3A* and *3B*). In contrast, in ZDF rats, PVAT exhibited significant inhibitory effect on neurogenic contractions (*Fig. 3A*) as well as on noradrenaline contractions (*Fig. 3B*).

When the group of ZDF rats was divided according to the level of their glycaemia, the individuals with high plasma glucose level (16 – 26 mmol/l) showed significantly increased contractions to electrical stimulation of sympathetic nerves in PVAT+ preparations, when compared to the individuals with lower values of plasma glucose (8.5 – 16 mmol/l) (*Fig. 4A*). The level of glycaemia within the ZDF rat group had no significant effect on exogenous noradrenaline contractions in PVAT+ preparations (*Fig. 4B*). The plasma glucose in ZDF rats had no significant influence on AUC values expressing contractions to endogenous and exogenous noradrenaline in PVAT– preparations.

DISCUSSION

In conditions of obesity, besides the visceral adipose tissue which is considered to be an important source of inflammation and other detrimental factors, the attention has recently started to be paid to perivascular adipose tissue (PVAT) (20). This fat depot is in tight contact with the vessel system and has more direct influence on its functional and structural properties. The presented experiments were based on the previous results of other authors who revealed the abnormal properties of PVAT in obesity which, when compared to the normal conditions, loses its anticontractile effect and has increased capacity of producing pro-contractile, inflammatory and atherogenic factors (14, 21). Xia and Li (12) have explained that this could be related to the changing quantitative and qualitative properties of PVAT during gaining of total body adipose mass.

In the present study, three rat strains with different levels of body adiposity were examined. As a high adiposity representative, Zucker diabetic fatty (ZDF) rats were used, being one of the key models in the research of obesity and metabolic syndrome, which develop as a result of impaired satiety reflex and chronic hyperphagia due to improperly coded leptin receptor gene in these rats (22). The presented results show that, besides strongly increased amount of retroperitoneal and epididymal fat, ZDF rats had higher relative liver and kidney weights, indicating the development of non-alcoholic fatty liver disease and associated kidney impairment (23-25). Impairment was confirmed also by the finding of increased lipid peroxidation in the liver tissue indicated by higher TBARS concentration comparing to Wistar rats; however, unexpectedly, in the left heart ventricle the oxidative stress markers were not significantly altered, and in the kidney tissue, conjugated diene concentration was even lower in ZDF comparing to Wistar rats. Altogether, the changes in oxidative stress markers in ZDF rats were not as substantial as expected, when compared with the findings of other authors (26-28). Therefore it seems that the lipid peroxidation within these organs might not be a reliable marker of the severity of metabolic syndrome-induced alterations in ZDF rat strain.

In our previous paper, increased systolic blood pressure of ZDF comparing to ZL rats was confirmed (29), which corresponds with the finding of the present study documenting higher relative heart weight in ZDF rats, probably as a compensatory response to the increased pressure load. According to the literary data, the blood pressure level in age-matched Wistar rats is similar to the values of ZL rat group (30).

The presented observations do not include the quantification of PVAT naturally occurring on superior mesenteric arteries in particular experimental groups, which is considered to be a limitation of the study. The examinations were focused on qualitative (vasomodulatory) properties of PVAT and the results indicate that in ZDF rats the presence of intact PVAT on isolated mesenteric arteries might have an important inhibitory effect on their sympatho-adrenergic contractions. Moreover, the modulatory influence of PVAT on arterial contractile responses was not in a direct proportion to the different level of total body adiposity seen in different rat groups used in this study. Previously, our research on healthy Wistar-Kyoto rats brought findings that moderate increase in body fat induced by high fructose administration enhanced the anticontractile effect of PVAT in their mesenteric arteries which might be responsible for attenuation of pathological increase in vascular tone during early phases of gaining weight (31). In agreement with Xia and Li (12) we assumed that in slightly overweight subjects the moderately increased PVAT might have protective and beneficial effect on vascular function, and could be associated with the observed “obesity paradox” in human studies documenting the positive effects of mildly increased BMI on cardiovascular functions in humans (32). However, after reaching certain level of adiposity, the properties of PVAT should importantly change due to inflammatory and diabetic alterations of this tissue; Xia and Li (12) described the central mechanisms underlying the PVAT dysfunction in obesity consisting of PVAT hypoxia, inflammation, and oxidative stress.

The observations of the presented study were focused on the manifestation of sympathetic activity in isolated mesenteric arteries and on its modulation by perivascular fat. Obesity is commonly associated with higher sympathetic vasomotor tone, which possibly contributes to increased cardiovascular risk. The increase in sympathetic activity was detectable also in the overweight condition, suggesting that the adrenergic overdrive associated with body fat increase exhibits an early manifestation and progression with the increased severity of these conditions (3). In the present study, it was assumed that with sharply increasing body adiposity among the different rat strains used, their PVAT would have more detrimental and procontractile effect, and could contribute to the increased arterial sympathoadrenergic activity, as indicated in the review of Saxton *et al.* (33). However, the presented results on isolated mesenteric arteries indicate rather enhanced anticontractile influence of mesenteric PVAT in ZDF rats. Interestingly, the observations of Schreihofer *et al.* (34) showed that obesity in Zucker rats is characterised by elevated sympathetic tone but also by remodelling of sympathoadrenergic reactivity in cardiovascular system to favour higher perfusion of the mesenteric circulation and lower perfusion of limbs. In their study, obese rats displayed enhanced adrenergically mediated vasoconstriction in the hindquarters; in contrast, the responses were reduced in the mesenteric circulation. When determining the adrenergic pressor reactivity in the whole vascular bed of rats *in vivo*, it might be supposed that PVAT has similar effect like in isolated arteries with intact perivascular fat; therefore, the decreased adrenergic contractions in ZDF mesenteric arteries observed in the presented *ex vivo* study are in keeping with the findings of Schreihofer *et al*. (34). In fact, however, our observations show that after PVAT removal, mesenteric arteries from ZDF rats had significantly enhanced contractions to endogenous as well as to exogenous noradrenaline, demonstrating increased sympathetic innervation of arteries as well as higher sensitivity of their adrenoreceptor system comparing to Wistar or ZL rats. These results indicate that PVAT around mesenteric arteries might be responsible for the decreased manifestation of sympathetic activity in mesenteric vascular bed of this obese, hyperphagic model. Exaggerated production of nitric oxide or hydrogen sulphide might partially explain this increased anticontractile effect of mesenteric PVAT in obese rats (35, 36). It can be presumed that in ZDF rats the mesenteric arterial bed is somewhat specific, possibly due to the effect of hyperphagia. In this rat strain, increased food intake may cause sustained nutrient-induced increase in intestinal blood flow which might be enhanced also by anticontractile influence of their expanded mesenteric PVAT, as indicated by our results. Moreover, the structural alterations in mesenteric vascular bed including the altered diameter of arteries were found to contribute to maintaining higher mesenteric blood flow in obese Zucker rats (37).

When translating the present observations into human medicine, potent sympathoinhibitory effect of mesenteric PVAT documented in this study could participate in the autonomic disturbances in predisposed subjects, leading to cardiovascular dysregulation and development of a clinically relevant postprandial fall in blood pressure known as postprandial hypotension (38). Sequeira and Rosario (39) demonstrated that in young obese adults the postprandial fall in blood pressure was significantly higher than in non-obese persons, suggesting that obese patients might be more prone to postprandial hypotension. From the presented results it follows that the expanded mesenteric PVAT attenuating the sympathetic vasoconstriction could contribute to this pathological state.

The obtained results show, furthermore, that in ZDF rats with high glycaemia (> 16 mmol/l) the contractions to sympathoneural stimulation in mesenteric arteries with preserved PVAT were significantly enhanced when compared to ZDF individuals with lower values of plasma glucose (8.5 – 16 mmol/l). This indicates that diabetic alterations in PVAT could reduce its capacity to inhibit the sympathoadrenergic contractions in mesenteric vascular bed. The negative effect of high glycaemia on PVAT anticontractile capacity was confirmed also in other diabetic rat models (40). Decreased level of hydrogen sulphide production within PVAT (41, 42) as well as impaired vascular nitric oxide

production (43) in diabetic rats could partially elucidate the observed reduction in PVAT anticontractile effect.

On the other hand, the presented findings document no anticontractile influence of PVAT in superior mesenteric arteries from healthy normotensive Wistar rats and ZL rats. In Wistar rats, ranging in their adiposity level between ZL and ZDF rat strains, neurogenic contractions in arterial preparations with preserved PVAT were even higher comparing to those without PVAT. ZDF rat arteries with preserved PVAT exhibited similar contractile responses when compared to those from Wistar rats; however, in PVAT-removed preparations, ZDF rats had significantly increased contractile responses to sympathoneural stimulation. These findings indicate important differences between these two rat strains in the interaction of PVAT and sympathetic nerves within superior mesenteric artery. ZDF rats seemed to have higher sympathetic innervation together with enhanced sensitivity to noradrenergic stimulation in mesenteric arteries; however, their increased adrenergic responses were specifically blunted with PVAT anticontractile effect. On the contrary, Wistar rats had decreased adrenergic sensitivity and sparser innervation of mesenteric arterial smooth muscle; however, richer content of sympathetic nerve terminals in mesenteric PVAT might overlap its potential anticontractile effect, causing the adrenergic contractions being even higher in arteries with preserved PVAT, as described previously in abdominal aorta (44).

In conclusion, the presented results show that the anticontractile effect of mesenteric PVAT might not be reduced in rats with increased adiposity; however, it might rather be influenced by the strain-specific distribution of sympathetic nerves between PVAT and the proper mesenteric arterial wall. Moreover, in hyperphagic ZDF rats characterized by enhanced arterial sympathetic tone, the sympathoinhibitory effect of mesenteric PVAT may be specifically associated with the increased nutrient-induced vasodilatation in mesenteric vascular bed, and could be negatively affected by diabetes.

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Conflicts of interest: None declared.

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