Exercise enhances vasorelaxation in experimental obesity associated hypertension

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Abstract

Objective: Regular exercise is recommended for the non-pharmacological treatment of hypertension, but the mechanisms underlying the lowering of blood pressure remain controversial. Therefore, we studied the effects of 22-week-long training on blood pressure, arterial reactivity, and metabolic abnormalities in a model of genetic obesity and moderate hypertension. Methods: Obese and lean Zucker rats were subjected to treadmill exercise from 8 to 30 weeks of age. Blood pressures were measured by the tail-cuff method, and urine was collected in metabolic cages. At the end of the study, the samples for biochemical determinations were taken, and reactivity of isolated mesenteric and carotid arterial rings was examined in standard organ chambers. Results: The exercise prevented the elevation of blood pressure which was observed in non-exercised obese Zucker rats, and also reduced blood pressure in the lean rats. The relaxations of norepinephrine-preconstricted mesenteric and carotid arterial rings to acetylcholine and nitroprusside were clearly improved by exercise in the obese rats. In the lean rats exercise enhanced vasorelaxation to nitroprusside in the mesenteric and carotid rings, and to acetylcholine in the carotid preparations. The exercise-induced improvement of endothelium-mediated dilatation to acetylcholine was abolished by nitric oxide synthesis inhibition with N\textsuperscript{G}-nitro-L-arginine methyl ester, but not by cyclooxygenase inhibition with diclofenac or functional inhibition of endothelium-dependent hyperpolarization by precontractions with KCl. The urinary excretion of the systemic prostacyclin metabolite (2,3-dinor-6-ketoprostaglandin F\textsubscript{1\alpha}) was increased two-fold by exercise in the obese and lean rats, whereas that of the thromboxane A\textsubscript{2} metabolite (11-dehydrothromboxane B\textsubscript{2}) remained unaffected. Treadmill training reduced blood glucose, cholesterol, and triglycerides, but did not affect the high levels of insulin in obese Zucker rats. Conclusions: These results suggest that the antihypertensive effect of long-term exercise in experimental obesity related hypertension is associated with improved vasodilatation. This is expressed as enhanced relaxation via endogenous and exogenous nitric oxide, and increased endothelial prostacyclin production. The improved control of arterial tone after training could be attributed to the alleviation of hyperlipidemia and insulin resistance, whereas hyperinsulinaemia per se remained unaffected.

Keywords: Endothelial factors; Exercise; Hypertension; Nitric oxide; Obesity

1. Introduction

The prevalence of hypertension is high among overweight subjects [1], and it often clusters with metabolic derangements including insulin resistance, hyperinsulinaemia, non-insulin-dependent diabetes mellitus and dyslipidemia [2]. Today regular physical activity is recommended as a non-pharmacological measure for the
treatment of hypertension [3], but whether exercise has a blood pressure-lowering action which is independent of its beneficial influence on body weight is not known. Even the antihypertensive mechanism of exercise remains obscure [1,4], although a reduction in arterial resistance has been suggested to account for the lowering of blood pressure by physical conditioning in essential hypertension [5]. In experimental animals exercise has been suggested to enhance vasodilatation via augmented endothelial release of nitric oxide (NO), prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF) [6–8], and also via increased sensitivity to relaxing stimuli in smooth muscle [9]. However, the majority of previous investigations about the vascular effects of training have been performed in normotensive animals [7,10–13], and only little information exists about the long-term effects of exercise on arterial function in experimental hypertension. Altogether the effects of chronic training on arterial tone remain far from clear, and many of the studies have provided contradictory results [6–9,12,13].

The Zucker fatty rat is a well-established experimental model of genetic obesity with autosomal recessive homozygous inheritance (fa/fa), the heterozygous and missing fa gene (Fa/?) producing the corresponding slender control strain, the lean Zucker rat [14]. The fatty mutation is characterized by insulin resistance, hyperinsulinaemia, glucose intolerance, hyperlipidaemia [14], and by gradual development of moderate hypertension [15]. In order to test the hypothesis whereby alterations in the control of arterial tone could participate in the antihypertensive effect of long-term physical exercise in obesity associated hypertension, we examined the reactivity of isolated mesenteric and carotid arterial rings in obese Zucker rats which were treadmill-trained for 22 weeks. The metabolic abnormalities of the animals were also addressed.

2. Methods

2.1. Animals and experimental design

Male obese and lean Zucker rats (Iffa Credo, France) were divided into four groups of equal systolic blood pressures; treadmill-exercised lean and obese rats (n=11 in both), and sedentary lean and obese rats (n=14 in both). The rats were housed two to three animals to a cage at 22°C (12-h light–dark cycle) with free access to food (R3 rat chow, Ewos, Södertälje, Sweden) and water. The exercised groups ran on a treadmill during early afternoon hours 5 days a week. The initial running time (rat age 8 weeks) was 5 min at 20 m/min, whereafter it was extended 5 min each week up to 45 min/day. With this setting (900 m/day, 20 m/min, 5 days a week) the rats were exercised for further 14 weeks (rat age from 16 to 30 weeks). This program was designed to exercise the rats at 40–60% of their maximal aerobic capacity [16,17]. The systolic blood pressures were measured with the tail-cuff method at 28°C (Model 129 blood pressure meter, IITC Inc.) during morning hours. The rats were weighed weekly and housed twice in metabolic cages for 24 h urine collection. After 22 study weeks, food was withdrawn 4 h before the rats were anaesthetized with urethane (0.9–1.2 g/kg) and exsanguinated via carotid artery cannulation. Blood was collected into chilled tubes with or without EDTA, and the plasma and serum samples were separated and stored at −70°C. The tissue samples were excised, and the hearts and epididymal fat samples were blotted dry and weighed. The experimental design was approved by the Animal Experimentation Committee of the University of Tampere, Finland, and the investigation conforms with the Guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

2.2. Mesenteric and carotid arterial responses

Six successive sections of the mesenteric artery and four of the left carotid artery (3 mm long) were cut. The endothelium was removed from some rings and the preparations were suspended in an organ bath chamber in physiological salt solution as previously described [18,19]. The preload was set to 1.5 g which rendered maximal contractions in both obese and lean Zucker rats. All vascular rings from each rat were subjected to an individual pre-planned experimental protocol.

Endothelium-independent relaxations to nitroprusside (NP) and molsidomine (SIN-1) were determined in endothelium-denuded rings precontracted with 1 μmol/l nor-epinephrine (NE). The relaxations to NP were also studied in the mesenteric rings after preconstrictions with 50 mmol/l KCl in order to eliminate smooth muscle hyperpolarization [20]. The relaxations of mesenteric rings induced by the return of 1 mmol/l K⁺ after preconstrictions by Na⁺-K⁺-ATPase arrest via exposure to K⁺-free medium were investigated to evaluate the function of the smooth muscle sodium pump [18]. Endothelium-dependent relaxations to acetylcholine (ACh) and ADP were examined after preconstrictions with 1 μmol/l NE. The relaxations to ACh were also elicited in the presence of 3 μmol/l diclofenac, 0.1 mmol/l N⁶-nitro-L-arginine methyl ester (L-NAME), and 1 mmol/l L-arginine (inhibitors of cyclooxygenase and NO synthase, and substrate for NO synthase, respectively). The relaxations to ACh were also studied in rings precontracted by 50 mmol/l KCl. Vasocostrictions elicited by NE, 5-hydroxytryptamine (5-HT), and KCl (20–125 mmol/l, obtained by replacing NaCl equimolarly with KCl) were determined in endothelium-intact rings. The responses were also examined in the absence and presence of L-NAME and diclofenac.

All rings were allowed a 30-min recovery period between the responses. The preincubation time for diclofenac, L-NAME, and L-arginine was 30 min. All
relaxations were presented as percentage of preexisting contraction, and the contractile forces were related to tissue dry weight which was measured after drying the rings overnight at 100°C. The negative logarithm of the concentration producing 50% of the maximal contraction ($pD_2$) was determined in each ring.

2.3. Urine prostanoids, and blood glucose, insulin, and lipids

For the analysis of the urinary metabolites of systemic PGI$_2$ and thromboxane A$_2$ (TXA$_2$), 2,3-dinor-6-ketoprostaglandin F$_{1a}$ (2,3-dinor-6-keto-PGF$_{1a}$) and 11-dehydro-thromboxane B$_2$ (11-dehydro-TXB$_2$), respectively, were extracted from urine as previously described [21,22]. Commercial kits were used for the measurements of blood glucose (E. Merck AG) and serum insulin (rat insulin radioimmunoassay, Novo Nordisk). Plasma cholesterol, triglyceride and high density lipoprotein (HDL) were determined by enzymatic methods (Boehringer Mannheim).

2.4. Drugs and the analysis of results

The following drugs were used: ADP (Boehringer Mannheim), diclofenac sodium salt (Ciba-Geigy), NP (E. Merck), NE hydrogenartrate (Fluka Chemie), SIN-1 (GEA), and ACh chloride, l-arginine, 5-HT, and L-NAME (Sigma Chemical Co). Statistical analysis was by one-way ANOVA supported by the Bonferroni test for pairwise between-group comparisons. When data consisted of repeated observations at successive time points, ANOVA for repeated measurements was applied [19]. All results were expressed as mean±SEM, with $P<0.05$ considered significant.

3. Results

3.1. Blood pressure, animal data, and blood glucose, insulin, and lipids

The sedentary obese Zucker rats developed a moderate hypertension, which was prevented by the 22-week-long training. The systolic blood pressures of the lean control rats remained stable, while exercise slightly lowered blood pressure also in the lean animals (Fig. 1, Table 1). The treadmill running increased the heart to body weight ratio, and reduced heart rate, body weight and epididymal fat in both obese and lean rats. The chow consumption was comparable in the two obese rat groups and greater than in their lean controls throughout the study (Table 1).

The obese rats expressed marked hyperinsulinaemia despite unaltered blood glucose when compared with the lean controls. The training did not significantly affect insulin levels in either the obese or the lean rats, whereas blood glucose was reduced in both groups (Table 1). The non-exercised obese rats showed severe hyperlipidemia, plasma total cholesterol being about three-fold and triglycerides 15-fold higher than in the lean controls. Although the concentration of HDL was also elevated in the sedentary obese rats, its proportion of the total cholesterol remained comparable (up to 70%) to that in the lean controls. In the obese rats, the exercise lowered total cholesterol and triglycerides by approximately 50% and 70%, respectively, while the HDL to total cholesterol ratio was increased. Total cholesterol was also reduced by about 30% following the exercise in the lean rats, but the triglycerides and the HDL to total cholesterol ratio were not changed (Table 1).

3.2. Mesenteric and carotid arterial responses

The endothelium-independent relaxations induced by NP were impaired in the NE-preconstricted mesenteric arterial rings, but not in those preconstricted with KCl, in the sedentary obese rats when compared with lean controls (Fig. 2A, B). The difference in the relaxation elicited by SIN-1 between the obese and lean rats was not quite significant ($P<0.06$; Fig. 2C). However, no differences were observed in the relaxations of carotid arterial rings to NP and SIN-1 between the non-trained obese and lean rats (Fig. 3A, B). The treadmill exercise enhanced the relaxations induced by the two NO-donors, NP and SIN-1, in NE-preconstricted mesenteric (Fig. 2A, C) and carotid arterial rings (Fig. 3A, B) of both obese and lean Zucker rats. When compared with the relaxations in NE-preconstricted rings, the response to NP was still augmented in KCl-preconstricted rings of the obese rats following the
exercise (Fig. 2B), whereas under these conditions which prevent smooth muscle hyperpolarization [20] the response to NP in the trained lean animals did not differ from their sedentary controls (Fig. 2B). The dilatations induced by the readdition of K⁺ after the K⁺-free medium-induced precontractions, were bluntly in the mesenteric rings of the obese rats (Fig. 2D). Also the relaxations induced by K⁺ repletion were augmented in the mesenteric preparations as compared to their non-exercised controls (Fig. 2B). The dilatations induced by KCl in the mesenteric arteries of the sedentary obese rats were impaired when compared to the respective lean rats (Fig. 4C). Further addition of L-arginine induced a partial reversal of the inhibitory action of L-NAME upon the responses to ACh, the effect of which was more pronounced in both obese and lean trained groups than the sedentary controls (Fig. 4D).
Fig. 2. Relaxations to nitroprusside after precontractions with 1 µmol/l norepinephrine (A) and with 50 mmol/l KCl (B), to molsidomine (SIN-1) after 11 precontractions with 1 µmol/l norepinephrine (C), and to the readdition of 1 mmol/l K after precontractions caused by exposure to K−-free medium (D) in isolated endothelium-denuded mesenteric arterial rings. The groups are as in Fig. 1. Symbols indicate mean±SEM, n=11–14 in each group, one vascular ring per animal. * P<0.05, ANOVA for repeated measurements.

The maximal contractile forces of mesenteric arterial rings induced by NE, 5-HT, and KCl were lower in the non-exercised obese animals, while these responses were increased by running so that the contractions of the trained obese group no more differed from those of the lean controls (Table 2). The vascular rings of the obese rats showed higher sensitivity (i.e. pD₂ value) to NE and 5-HT than those of the lean controls, while no differences in sensitivity to KCl were observed. Mesenteric vasoconstrictor sensitivity to NE was somewhat lower in the exercised when compared with the sedentary obese rats, and this difference was abolished by diclofenac. Furthermore, in the presence of diclofenac or L-NAME no significant differences in constrictor sensitivity were observed between the four study groups. The exposure to K⁺-free medium induced comparable maximal force generation in the obese and lean controls, while this response was also higher in the trained obese group when compared with the sedentary obese rats. It is noteworthy that exercise was without significant effects on the vasoconstrictor responses of the mesenteric artery in the lean rats. The maximal contractions of carotid rings induced by NE were lower in the obese rats when compared with the lean controls in the absence and presence of diclofenac, while no significant differences were observed in the presence of L-NAME. Moreover, the maximal contractions of carotid rings induced by NE were increased in the obese and lean rats by training, an effect which was observed in both the
absence and presence of diclofenac and L-NAME. The contractile sensitivities to NE in the carotid rings were similar in all study groups (Table 2).

3.3. Urine prostanoids

The urinary excretion of 2,3-dinor-6-keto-PGF$_{1\alpha}$ was increased in the young (8-week-old), but not in the adult (30-week-old) sedentary obese Zucker rats when compared with the lean controls (Fig. 5A). Moreover, the higher excretion of 11-dehydro-TXB$_2$ in the obese rats was further enhanced during the follow-up period (Fig. 5B). Thus, the ratio of PGI$_2$ to TXA$_2$ metabolite excretion, which was normal in the young obese animals, was reduced in the 30-week-old obese rats (Fig. 5C). The training augmented the excretion of 2,3-dinor-6-keto-PGF$_{1\alpha}$ in both obese and lean rats, whereas that of 11-dehydro-TXB$_2$ remained unaffected (Fig. 5A, B). Hence, the excretion ratio of vasodilating versus constricting prostanoid metabolites was increased in both exercised groups when compared with the corresponding non-exercised rats (Fig. 5C).
4. Discussion

This study demonstrated that long-term exercise prevented the development of hypertension in the obese Zucker rat, an effect which was associated with enhanced vasorelaxation. Furthermore, the improved vasodilatation could especially be attributed to enhanced sensitivity to NO in arterial smooth muscle of the exercised rats. The present results agree with previous findings whereby the hindlimb vascular resistance of obese Zucker rats was reduced after training [23]. Furthermore, regular exercise has also been found to decrease peripheral arterial resistance in essential hypertension [5], but the mechanisms of exercise-induced alterations in the control of vascular tone are poorly understood.

In lean normotensive animals training has been found to augment endothelial NO release [24,25], and even a short training period in dogs has been suggested to augment coronary dilatation and increase aortic NO synthase expression [10]. However, contradictory reports have also been published, since 20-week-long training was not found to influence coronary vasodilator responses to NP and endothelium-mediated agonists in Yucatan miniature swine [12]. Furthermore, an 11-week-long training period has...
The mesenteric artery
Norepinephrine

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5-Hydroxytryptamine

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The carotid artery
Norepinephrine

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<tr>
<td>With diclofenac and L-NAME</td>
<td>2.1±0.2</td>
<td>3.6±0.3*</td>
<td>1.7±0.2</td>
<td>3.1±0.4*†</td>
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Table 2
Parameters of contractile responses of isolated arteries

\[\text{Table 2}\]

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\* Values are mean±SEM, \(n=11–14\) for each group. \(\text{LEAN}\) and \(\text{OBESE}\) indicate lean and obese Zucker rats, respectively. \(\text{E}\) indicates exercised groups. \(\text{L-NAME}\), \(N^\text{\textsuperscript{-\text{G}-nitro-l-arginine methyl ester}}\); \(\text{pD}_2\), the negative logarithm of the concentration producing 50% of the maximal response. The vascular rings had intact endothelium.

\† Endothelium-denuded.

\* \(P<0.05\) vs \(\text{LEAN}\).

\‡ \(P<0.05\) \(\text{E-OBESE}\) vs \(\text{OBESE}\), Bonferroni test.

even been reported to impair the relaxation of isolated coronary arteries to isoprenaline and vasoactive intestinal polypeptide in dogs [13].

The mesenteric artery was chosen for this study, since blood flow in the splanchnic area is not increased during physical effort, in contrast to the blood vessels directly subjected to exercise-induced flow and shear stress [26]. Thus, the functional changes in this artery could reflect the more generalized vascular adaptations induced by exercise. The responses of the carotid artery were also studied in order to examine whether the training would elicit more widespread changes in the control of arterial tone. The 22-week-long training enhanced endothelium-dependent relaxations in both mesenteric and carotid arteries of the obese rats. Since the enhanced relaxations to ACh were abolished by L-NAME, but not by diclofenac and preconstriction with KCl, exercise augmented endothelium-mediated vasodilatation via NO. The fact that competitive reversal by l-arginine of the L-NAME-induced inhibition of vasodilatation to ACh was more pronounced in the trained animals than their sedentary controls supports the notion that exercise beneficially influenced the function of the endothelial l-arginine/NO pathway. In addition, the vasodilatations induced by the exogenous NO donors, NP and SIN-1, were augmented in both mesenteric and carotid arteries of the trained rats. Therefore, in addition to the probable increases in endothelial NO release, enhanced sensitivity to NO in arterial smooth muscle following the exercise may largely explain the observed improvement of vasorelaxation in these animals. It is noteworthy that the exercised obese group exhibited greater relaxations to nitroprusside in both the mesenteric and carotid arteries than the sedentary lean group, whereby regular training appears to be more influential than obesity in regulating the sensitivity of arterial smooth muscle to NO.

The enhancing influence of training on vasodilatation was supported by the improved K⁺ relaxations in the mesenteric arteries of the exercised rats. This relaxation induced by the readdition of K⁺ after precontractions due to sodium pump arrest with K⁺-free medium can be
explain the enhanced vasorelaxations following regular exercise in obesity associated hypertension.

It is noteworthy that the present exercise program enhanced vasorelaxations to NP, SIN-1, and K⁺ return also in the lean rats. Most of the earlier reports have suggested that the improved vasodilatation following physical conditioning is endothelium-mediated, whereas the endothelium-independent dilatations have remained unaffected [24,25]. The discrepancy between the present and previous results can be attributed to the differences of the vascular regions studied, and to the duration of the exercise period. Prolonged training may be required to achieve adaptive changes in arterial smooth muscle, while endothelial function may be more readily affected already after shorter periods of physical activity [10]. The exercise-induced vascular adaptations have been reported to deviate even along one arterial bed such as the coronary circulation [6].

In this study, the impaired vasodilatation to ACh in the mesenteric rings of the sedentary obese rats could be attributed to reduced endothelium-dependent hyperpolarization of smooth muscle, since the functional inhibition of EDHF by depolarization with KCl abolished the difference in the relaxations to ACh between the non-exercised obese and lean rats [20]. The relaxations to NP were also diminished in the NE-precontracted mesenteric arteries of the obese rats, while the elimination of smooth muscle hyperpolarization by KCl abolished also the difference in response to NP between the sedentary obese and lean rats. Therefore, impaired hyperpolarization of smooth muscle appears to play a significant role in the attenuated mesenteric arterial vasodilatory responses of the sedentary obese Zucker rats. In contrast, in the carotid artery the relaxations to ACh and NP were not impaired in the obese rats, and the response induced by ACh appeared to be largely mediated via NO in all groups, since it was completely abolished by L-NAME. Altogether the present results indicate that endothelium-dependent relaxation in the mesenteric artery was mediated by NO, EDHF and vasodilatory prostanoids, whereas in the carotid artery the response appeared to be mediated by NO alone. The high contribution of EDHF to the endothelium-dependent vasodilatation (reflected as the L-NAME- and diclofenac-resistant relaxation) in the mesenteric artery of the lean rats may provide an explanation to the finding that regular exercise did not improve the relaxation to ACh in these animals, although the response to exogenous NO was clearly enhanced.

The training increased the urinary excretion of 2,3-dinor-6-keto-prostaglandin (PG) F₁₀, a major metabolite of PG₁₂, produced by the endothelium. The excretion of this metabolite also reflects the amount of PG₁₂ released into the systemic circulation [28]. Thus, endurance training enhanced PG₁₂-mediated endothelial vasodilatory function, the mechanism of which also provides an explanation to the lowering of blood pressure. More importantly, this finding demonstrates that vasodilatory endothelial autacoid production
was increased in the exercised rats. The sedentary obese Zucker rats showed exaggerated production of the vasocostricting TXA₂, since the urinary excretion of its metabolite 11-dehydro-TXB₂ was increased. This corresponds with previous findings in human diabetes mellitus [29]. The increased excretion of TXA₂ metabolite was further accelerated during aging, thereby leading to a vasocostriction-favouring imbalance between the prostanoi ds in the non-exercised obese rats. It is noteworthy that the PGI₂ to TXA₂ metabolite excretion ratio was normalized by exercise, i.e. it was similar in the sedentary lean and exercised obese rats.

High blood lipids and oxidized low-density lipoprotein (LDL) are mediators of vascular dysfunction in hypercholesterolaemia [30]. However, almost all of the plasma triglyceride and cholesterol content in Zucker rats resided in HDL and very low-density lipoprotein, and not in LDL, suggesting that oxidized LDL could not explain the changes in vasodilatation in the present study. Although the role of triglycerides in regulation of vasomotion remains poorly characterized, hypertriglyceridemic patients express diminished vasodilatation of skin vessels, the impairment of which is reversed by the lowering of triglycerides [31]. Furthermore, cutaneous vasodilatations due to ACh, isoproterenol and NP have been shown to negatively correlate with triglyceride levels and positively with HDL to total cholesterol ratios in man [32]. Therefore, in the present study the exercise-induced decrease in triglycerides and increase in HDL to total cholesterol ratio together with improved arterial dilatation suggest that the correction of vascular dysfunction in the obese Zucker rats by training may be mediated by its antilipemic action.

The blood glucose-lowering influence of exercise despite unaltered insulin levels in the present investigation indicates that insulin resistance was alleviated by training, which agrees with previous findings in Zucker rats [33]. An imbalance between the pressor (sympathetic nerve stimulation, antinatriuresis, vascular hypertrophy) and depressor (vasodilatation) actions of insulin has been proposed as a link between insulin resistance and hypertension [34]. According to this hypothesis there is a resistance to the actions of insulin on glucose uptake and vasodilatation, but not to its pressor activities [34]. The vasodilatory mechanisms of insulin include the stimulation of Na⁺,K⁺-ATPase [35], K⁺ channels and endothelial NO production [36]. Thus, impaired vasodilatation in the sedentary obese Zucker rats in this study could have reflected their insulin resistant state, the alleviation of which could partially explain the improved vasorelaxations after exercise. Since hyperinsulinaemia in the obese rats was not affected in spite of the antihypertensive and vasodilatation-enhancing effects of exercise, the present results suggest that high insulin level per se was not a key determinant for the elevated blood pressure and vascular dysfunction in obesity associated hypertension. Furthermore, although hyperglycemia has been shown to suppress arterial dilatation and predispose to the development of hypertension [37], the lack of overt basal hyperglycemia in the obese Zucker rats suggests that the modest changes of blood glucose were unlikely to explain the alterations of arterial reactivity.

In conclusion, the present results suggest that regular long-term exercise is associated with enhanced vasodilatation in the obese Zucker rat. This is expressed as increased arterial sensitivity to both endogenous and exogenous NO, increased release of prostacyclin from the vascular endothelium, and possibly also as augmented sodium pump action in arterial smooth muscle. Improved arterial function following physical training in experimental obesity associated hypertension may be mediated via alleviation of hyperlipidemia and insulin resistance, whereas hyperinsulinaemia per se does not seem to play a key role.

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References


