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Original article

Effect of high free fatty acids on the anti-contractile response of perivascular adipose tissue in rat aorta

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ABSTRACT

To determine whether high free fatty acids (FFA) could affect the anti-contractile properties of perivascular adipose tissue (PVAT) in rat aortas. Wistar rats were divided into normal, obesity and fenofibrate groups and fed a normal, high-fat, and high-fat plus fenofibrate diet, respectively. Thoracic aortas with or without PVAT (PVAT+ and PVAT−) were prepared with either intact endothelium (E+) or with endothelium removed (E−). Aortas pretreated with either 500 μmol/L of palmitic acid (PA) or physiological salt solution (PSS), as a control, were used for *in vitro* study. Concentration-dependent responses of aortas to norepinephrine were measured. The anti-contractile effects of PVAT were attenuated in both obese rats with high FFA levels and in the PA group in the presence of endothelium, but not in the absence of endothelium. The attenuation of the anti-contractile effect was restored by reducing FFA levels in the fenofibrate group ($P < 0.05$). Incubation of aortas (PVAT+ E+) with nitric oxide (NO) synthase inhibitor and tumor necrosis factor-alpha (TNF-α) in the normal group caused attenuation of the anti-contractile effect of PVAT ($P < 0.05$). Incubation of aortas (PVAT+ E+) in the obese and PA groups with a NO donor, anti-TNF-α antibodies or free radical scavengers partially restored the anti-contractile effect of PVAT ($P < 0.05$). Under both acute and chronic conditions, high FFA levels could attenuate the anti-contractile properties of PVAT by an endothelium-dependent rather than an endothelium-independent mechanism, in which inflammation and oxidative stress may play important roles.

1. Introduction

Obesity is a major health problem and is strongly associated with the onset and development of ischemic cardiovascular disease in which vascular injury occurs [1,2]. Endothelial dysfunction, from reduced endothelium-dependent relaxation responses to acetylcholine, is usually seen in obese subjects and is regarded as the early manifestation of vascular injury [3]. Studies have suggested that endothelial dysfunction is caused by several risk factors in obesity, of which elevated free fatty acid (FFA)-induced endothelial nitric oxide (NO) bioavailability is the leading factor [4,5].

Recent studies have reported the importance of perivascular adipose tissue (PVAT), which is situated outside the adventitial layer and surrounds almost all systemic blood vessels, in the modulation of vascular function and has some similarity to endothelium [6–8]. PVAT significantly attenuates vasoconstrictive responses to norepinephrine or phenylephrine in various vessels by releasing various unknown relaxation factors, called PVAF-derived relaxing factors [9,10]. This anti-contractile response of PVAT involves both endothelium-dependent and endothelium-independent mechanisms through NO release or hydrogen peroxide production [11]. However, further studies have found that PVAT-related anti-contraction was impaired in some conditions. Gao et al. [7,12]. reported that PVAT-associated inhibition of vessel contraction was reduced in spontaneously hypertensive rats and enhanced in streptozotocin-induced diabetic rats. As PVAT mass increases in obese subjects, one would expect increased PVAT to have a protective effect on vessels due to increased PVAF-derived relaxing factors being released. However, the anti-contractile response of PVAT is lost in obese subjects [13,14]. Greenstein et al. [15]. reported that local inflammation abolishes the protective anti-contractile properties of PVAT in obese patients. Further studies indicated that the PVAT-induced anti-contractile response is not adipose-mass dependent but is associated with PVAT function regulated by various adipokines [16].

Although it is known that the anti-contractile properties of PVAT are lost in obese patients and animal models [15], the underlying mechanisms of this phenomenon are not clear. High FFA levels are a common feature of obesity, and it has been suggested that excessive FFA could cause impaired endothelium-dependent vasodilation

Abbreviations: Hs-CRP, high-sensitivity C-reactive protein; FFA, free fatty acidsMDA, malondialdehyde; NO, nitric oxide; PVAT, perivascular adipose tissue; PA, palmitic acid; PSS, physiological salt solution; eNOS, endothelial nitric oxide synthase.

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through an inflammatory pathway and oxidative stress in obesity subjects [5]. It is therefore logical to hypothesize that PVAT dysfunction seen with obesity is also related to elevated FFA levels. The aim of the present study was to determine whether high FFA levels could affect the anti-contractile properties of PVAT in rat aortas, and if so, to investigate the potential mechanisms involved.

2. Materials and methods

2.1. Experimental animals

Six-week-old male Wistar rats (specific pathogen-free) were purchased from the Experimental Animal Center of Weifang Medical University (Weifang, China) and randomly divided into a normal group, an obesity group and a fenofibrate group (to reduce FFA levels). Rats in the normal group were fed a regular diet (11.71% fat, 65.06% carbohydrate, 23.23% protein, 330 kcal/100 g). Rats in the obesity group were fed a high-fat diet (50.10% fat, mainly saturated, 33.60% carbohydrate, 16.30% protein, 493 kcal/100 g). Rats in the fenofibrate group were fed a high-fat diet plus fenofibrate (100 mg/kg/d) [17]. All groups were housed under standard laboratory conditions with unrestricted access to water and food. After 8 weeks, rats were anesthetized by intraperitoneal administration of sodium pentobarbital (60 mg/kg) and intact thoracic aortas were immediately collected for vascular reactivity and PVAT area measurements. Blood samples were collected and separated for plasma glucose, triglyceride (TG), total cholesterol (TC), FFA, high-sensitivity C-reactive protein (hs-CRP) and malondialdehyde (MDA) measurements.

The use of rats for this study was approved by the Institutional Animal Care and Use Committee and the study conformed to the Guide for the Care and Use of Laboratory Animals.

2.2. Plasma measurements

Plasma glucose and plasma hs-CRP concentrations were measured using the glucose oxidase method and commercially available ELISA kits (USCN Life Science, Wuhan, China), respectively. Plasma FFA, TG, TC and MDA levels were measured by colorimetric assays.

2.3. Assessment of aortic vascular reactivity

Aortic vascular reactivity was determined as described previously [7]. Aortas were immersed in ice-cold oxygenated physiological salt solution (PSS) containing 119 mM NaCl, 25 mM $NaHCO_3$, 5.5 mM glucose, 4.7 mM KCL, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.6 mM $CaCl_2$ and 0.026 mM EDTA in preparation for assessment of aortic vascular reactivity. The aortic rings (3 mm in length for each) from the three groups with or without PVAT (PVAT+ and PVAT−) were prepared with either intact endothelium (E+) or with endothelium removed (E−). The endothelium was removed mechanically by inserting a roughened stainless-steel wire into the lumen and gently rolling the vessel segment on damp filter paper. Successful removal of endothelium was confirmed by there being no relaxation effect in response to acetylcholine. Rings were threaded onto steel wires and immersed in chambers containing 20 ml volume of PSS (95% oxygen and 5% carbon dioxide) at 37 °C. The steel wires were connected to a force transducer linked to a data acquisition system (PowerLab, AD Instruments, Castle Hill, Australia) to record force changes. After being at equilibrium for 60 min, rings were challenged with 60 mM KCL twice until a sustained contraction was achieved in each ring. The cumulative concentration-dependent responses to norepinephrine (10^{-9}–10^{-5} M) (Sigma, St. Louis, MO, USA) were expressed as a percentage of the contraction to KCL.

2.4. Mechanism study

To study the effects of high FFA levels on PVAT-derived relaxing factors, PVAT+ aortas were used as a donor and PVAT– aortas used as a recipient. Aortas were pre-constricted with norepinephrine (1 uM) and then the solution from PVAT+ aortas was transferred to an organ bath containing PVAT– aortas from obese rats. The subsequent contractile response to norepinephrine was recorded. The effects of incubating aortas (PVAT+ E+) of normal rats with NO synthase inhibitor NG-mono-methyl-L-arginine (L-NMMA, 5×10^{-5} mmol/L, Sigma) or tumor necrosis factor-alpha (TNF-α, 0.001 ug/ml) and incubating aortas (PVAT+ E+) of obese rats with NO donor (MAHMA NONOate, 100 umol/L, Sigma) [11], anti-TNF-α antibodies (infliximab, 3.4×10^{-4} M, for 30 min, Sigma) or free radical scavengers, superoxide dismutase and catalase (80 and 120 U/ml, for 30 min, Sigma) [14], on anti-contractile responses were determined.

2.5. In vitro study of the anti-contractile effects of PVAT

To study the acute effects of high FFA on the anti-contractile effects of PVAT, aortas from normal rats were pre-treated with 500 μmol/L [18–20] palmitic acid (PA) or with physiological salt solution (PSS) as a control. The concentration-dependent responses of aortas to norepinephrine were studied in an organ bath.

To study the effects of high FFA levels on PVAT-derived release factors, PVAT+ aortas were used as a donor and PVAT– aortas were used as a recipient. Both aortas from the same rat were first pre-constricted with norepinephrine (1 uM) and then the solution from PVAT+ aortas incubated with PA was transferred to an organ bath containing the PVAT– aorta. The subsequent contractile response to norepinephrine was recorded.

To study the mechanism of FFA-induced attenuation of the anti-contractile response of PVAT, the effects of incubation with NO donor (MAHMA NONOate), anti-TNF-α antibodies (infliximab) or free radical scavengers on the anti-contractile response of PVAT in aortas were determined.

2.6. Morphometric measurement of PVAT

Aortas were fixed in formalin and stained with hematoxylin and eosin. PVAT areas and adipocyte areas were measured by microscopy with Image-Pro Plus 6.0 software (Media Cybernetics, USA).

3. Results

3.1. Biometric and blood parameters

Rats from both the obesity and the fenofibrate groups exhibited increased body weight, visceral fat, PVAT areas and adipocyte areas in aortas compared with the normal group. No significant differences were observed in the body weight, visceral fat, PVAT areas or adipocyte areas between the obesity and the fenofibrate groups. Plasma FFA, TG, hs-CRP and MDA levels in the obesity group were significantly higher than those of the normal group ($P < 0.05$ for all measurements). Fenofibrate intervention decreased plasma FFA levels by 44.6% ($P < 0.05$) and also reduced plasma TG, hs-CRP and MDA levels ($P < 0.05$). There were no significant differences in plasma glucose or TC levels between any of the experimental groups ($P > 0.05$) (Table 1). There were also no significant differences in blood pressure between the experimental groups (normal control: 124.75 ± 11.46 mm Hg; obesity: 131.97 ± 22.17 mm Hg; fenofibrate: 129.13 ± 20.58 mm Hg; $P > 0.05$).