Altered arachidonic acid metabolism impairs functional vasodilation in metabolic syndrome

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Submitted 26 April 2005; accepted in final form 8 September 2005

IT IS WELL ESTABLISHED THAT, during periods of increased skeletal muscle metabolism (such as during exercise), blood flow to the muscle increases (functional hyperemia) because of dilation of local arterioles (functional dilation), and this vasodilatory response is dependent, in some part, on endothelial function (22). However, in patients and animals with metabolic syndrome, endothelial dysfunction may occur early in the pathology of this syndrome (4), possibly contributing to the impaired functional hyperemia seen in metabolic syndrome. For example, patients with type 2 diabetes exhibit an impaired endothelial-dependent vasodilatory response to ACh and a significant impairment of the functional hyperemic response to leg cycle ergometer exercise (14). Our laboratory and others (8, 32) have demonstrated that the obese Zucker rat (OZR), a model of metabolic syndrome, have both an attenuated functional and ACh-induced vasodilation in skeletal muscle arterioles. Although the mechanism(s) responsible for the impaired functional dilation remains unclear, these studies reveal a possible relationship between the endothelial dysfunction and the impaired functional vasodilation.

The determination of the mechanism(s) responsible for the impaired functional dilation is important for treatment of metabolic syndrome. The endothelium is a prominent source of nitric oxide (NO) and prostaglandins in the vasculature (3). Several previous studies have emphasized the role of altered NO synthase expression (21) or reduced NO bioavailability (6) in the endothelial dysfunction in metabolic syndrome, which results in an attenuated endothelium-dependent vasodilation (9, 12). However, these studies did not test the vasodilatory response during muscle contraction. Indeed, the role of NO in mediating functional vasodilation in humans and animals is still unclear. Our previous study showed that, in the cremaster muscle, inhibition of cyclooxygenase with indomethacin significantly attenuates functional dilation (19, 24), suggesting that prostaglandins play a central role in functional hyperemia (10). Indeed, endothelial-derived prostacyclin (PGI2) has been proposed as playing an important role in regulating local blood flow during exercise through activation of PGI2 receptors prostacyclin (IP) on vascular smooth muscle cells (20, 30).

In addition to the arachidonic acid (AA)-derived vasodilators such as PGI2 and eicosanoids, vasoconstrictor metabolites of AA may also regulate vascular tone. For example, PGH synthase catalyzes the conversion of AA to PGH2, from which PGI2 and thromboxane A2 (TXA2) are derived. TXA2 and its precursor, prostaglandin H2 (PGH2), can activate TP receptors on vascular smooth muscle cells causing vasoconstriction. Under normal conditions, PGI2 generation is a major pathway of AA metabolism during functional hyperemia, and TXA2 has been found to decrease during exercise in an exercise-intensity-dependent manner (13). However, in both patients and animal models of metabolic syndrome, there is an elevated TXA2-to-PIG1 ratio in urine and blood plasma (11, 18), suggesting an altered AA metabolism, which we hypothesize may contribute to the impaired functional vasodilation seen in metabolic syndrome. Our previous study showed that 12-wk-old OZRs have increased body weight, fasting blood glucose, and insulin levels compared with the lean controls. Although 12-wk-old OZRs are not hypertensive, ACh-induced endothelium-dependent and functional vasodilation are impaired in this experi-

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Arachidonic acid metabolites and functional hyperemia

Mendal model (32). The present study was designed to test the hypothesis that the impaired functional dilation in this model of metabolic syndrome results from an increased TP-mediated vasoconstriction and/or a decreased PGI2-induced vasodilation.

**METHODS**

**Animals.** Twelve-week-old OZRs and lean Zucker rats (LZRs) were acquired from Harlan Laboratories (n = 22; 11 LZRs and 12 OZRs). The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were carried out according to both the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the guidelines of the Animal Welfare Act. All of the rats were housed two or three animals per cage at 22°C (12:12-h light-dark cycle) with free access to regular food and water.

**Microcirculatory surgical preparation.** The right spinotrapezius muscle was prepared for experimental observation, as previously described (17). In brief, rats were anesthetized with pentobarbital sodium (65 mg/kg ip), and the trachea was intubated. Animals spontaneously breathed a gas mixture containing 30% oxygen and 70% nitrogen. At all times during the surgery and subsequent experiment, the spinotrapezius muscles were kept at in situ dimensions and continuously superfused with a physiological salt solution (in mM: 118.07 NaCl, 6.17 KCl, 2.55 CaCl2, and 25 NaHCO3), which was aerated with a 5% CO2-95% N2 gas mixture (pH 7.4, 35°C). All chemicals were purchased from Sigma (St. Louis, MO). During the protocol, animals did not recover from anesthesia. When the experiment was finished, animals were euthanized by a cardiac injection of 10% potassium chloride. Death was confirmed by a lack of heart beat and spontaneous breathing.

**Experimental measurements.** The microcirculation of the spinotrapezius muscle was transilluminated and observed with a Nikon 10% potassium chloride. Death was confirmed by a lack of heart beat and spontaneous breathing. After the vessel had no effect on the vasodilatory response to muscle contraction solution to determine whether the previous dilations were maximal.

**Analytic and statistical methods.** Arteriolar diameter data were collected at 1 Hz using a computer equipped with a Computer Boards 8-bit analog-to-digital converter and stored to disk for later analysis. Data were analyzed using two-way, repeated-measures ANOVA. Where significant main effects occurred, individual groups were compared using the Holm-Sidak method. All data are means ± SE. A probability of P < 0.05 was accepted as statistically significant for all comparisons.

**RESULTS**

**Basal and maximal diameters.** The arteriolar basal diameters and the vasodilatory responses to adenosine (10 μM) are presented in Fig. 1. Neither the resting diameters nor the adenosine-induced dilations were significantly different between LZRs and OZRs.SQ-29548 (1 μM) did not affect resting diameters (data not shown).

**Functional vasodilation.** Figure 2 presents the vasodilatory response to muscle stimulation of arcade arterioles in the spinotrapezius muscle from LZRs and OZRs. In all cases, muscle contraction resulted in a significant increase in luminal diameter. The functional dilatation was significantly blunted in OZRs compared with their lean controls. SQ-29548 treatment had no effect on the vasodilatory response to muscle contraction in LZRs, whereas it significantly enhanced the vasodilatory response in OZRs. However, the vasodilatory response to muscle stimulation in OZRs in the presence of SQ-29548 was still significantly less than that in LZR. Superfusion with 10 μM adenosine resulted in a greater increase in diameter compared with the vasodilatory responses to muscle contraction (data not shown).

**Iloprost-induced vasodilation.** The vasodilatory responses to topical administration of iloprost are presented in Fig. 3. The administration of iloprost resulted in a concentration-dependent increase in luminal diameter in animals from both groups. However, vasodilation in response to iloprost was significantly

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**Fig. 1.** Basal diameter and adenosine-induced vasodilation in spinotrapezius arterioles from lean (LZRs) and obese Zucker rats (OZRs). There is no significant difference in the basal or maximal diameters between LZRs and OZRs (n = 6/group).
The improvement in the vasodilatory responses in the presence of SQ-29548 treatment partially restores the vasodilatory response to muscle stimulation from OZR. *LZR vs. OZR within control, \( P = 0.002 \); +control vs. SQ-29548 within OZR, \( P = 0.036 \); #LZR vs. OZR within SQ-29548, \( P = 0.03 \) (n = 5 for LZR and n = 6 for OZR).

reduced in OZRs at all concentrations compared with their lean controls. SQ-29548 treatment had no effect on the vasodilatory responses to iloprost in either group (data not shown).

AA-induced vasodilation. Figure 4 presents the arteriolar vasodilatory responses after addition of AA to the superfusion solution. AA resulted in a significant increase in luminal diameter; however, the vasodilatory response was significantly attenuated in OZRs compared with their lean controls. SQ-29548 treatment significantly enhanced the vasodilatory response in OZRs but had no effect in LZRs. In the presence of SQ-29548, the vasodilatory response to AA in OZR was still significantly less than the vasodilatory response in LZRs.

SNP-induced dilation. The vasodilatory response to SNP is presented in Fig. 5. The administration of SNP resulted in a concentration-dependent increase in luminal diameter in animals from both groups. However, the vasodilatory responses to 1 and 10 \( \mu \)M SNP were significantly reduced in OZRs compared with LZRs. SQ-29548 treatment had no effect on the vasodilatory responses to SNP in either group (data not shown).

DISCUSSION

The major findings of this study are that 1) functional hyperemia is impaired in OZR, and the TP receptor antagonist SQ-29548 partially restores the vasodilatory response to muscle contraction in the OZR, with no effect in LZR (Fig. 2); 2) vasodilation in response to iloprost was attenuated in OZRs compared with LZRs (Fig. 3); and 3) AA-induced dilation was significantly blunted in OZR compared with LZR, and TP-receptor inhibition enhanced the vasodilatory response only in OZRs (Fig. 4). The improvement in the vasodilatory responses in the presence of SQ-29548 along with the impaired vasodilatory responses to iloprost suggest that the impaired functional dilation in OZR results from an increased TP-mediated vasoconstriction and decreased PGI2-induced vasodilation.

Numerous studies have demonstrated that the increase in blood flow in skeletal muscle during exercise is reduced in animal models (7) and humans (5) with metabolic syndrome. However, the mechanisms responsible for the impaired hyperemia are unclear. Consistent with the results of our previous work (32), the present study shows that the arteriolar dilation in response to muscle contraction is significantly blunted in 12-wk-old OZRs (Fig. 2). Similar results were also found in patients with type 2 diabetes (14). Because previous studies have supported a critical role of prostaglandin(s) in mediating functional vasodilation (10, 19, 20, 24, 30), it is possible that the impaired functional vasodilation in metabolic syndrome may be due to altered AA metabolism. Indeed, previous studies have shown that urinary thromboxane B2 excretion is enhanced in 12-wk-old OZRs associated with an increased cyclooxygenase-2 expression in kidney (3, 15).

Figure 2 shows that pretreatment with the TP receptor antagonist SQ-29548 improves the reduced functional hyperemia in OZR, suggesting that the impaired functional dilation is associated with an increased TP-mediated vasoconstriction. The vasodilatory response to AA is also blunted in OZR (Fig. 5). This is consistent with findings from Frisbee’s laboratory (8), who showed attenuated AA-induced dilation of isolated gracilis arteries from 13- to 15-wk-old OZRs. They suggested the impaired vasodilation in OZR is due to an inactivation of endothelium-derived prostacyclin by superoxide anion. In the
The present study, SQ-29548 treatment augmented the AA-induced dilation in OZR with no effect in LZR (Fig. 5), which directly supports the hypothesis that AA metabolism is altered in the OZR, resulting in an increased TP-mediated vasoconstriction. There is evidence that increased formation of ONOO⁻ in cultured human aortic endothelial cells exposed to high glucose results in inhibition of prostacyclin synthase and a shift in AA metabolism to the PGH₂ precursor PGH₂ or other TP receptor agonists (33). Therefore, it is possible that the increased TP-mediated vasoconstriction observed in the present study is secondary to an enhanced superoxide anion production. In addition, the finding that the administration of exogenous AA caused blunted vasodilatory responses in OZRs (Fig. 5) suggests that the impaired functional hyperemia is related to differences in AA metabolism rather than AA production in response to muscle stimulation. Further studies are needed to determine whether the altered AA metabolism in this experimental model is due to hyperglycemia and increased superoxide anion.

Basal production of TXA₂ from the vasculature in spinotrapezius may not be different between groups because SQ-29548 had no effect on basal arteriolar diameter in the present study. However, the vasodilatory responses to muscle contraction and AA in the presence of SQ-29548 were significantly increased in OZRs, suggesting an enhanced TP-mediated vasoconstriction in this setting. Because both PGH₂ and TXA₂ can activate the TP receptor, it is unclear whether the attenuated functional dilation is a result of an accumulation of PGH₂ or an elevated TXA₂ synthesis or both. For example, Traupe et al. (29) showed a twofold increase in thromboxane synthase expression in carotid artery of obese mice, whereas renal vasoconstrictor responses to AA in the diabetic rat were reduced by thromboxane A₂/prostaglandin H₂ receptor antagonism but not by inhibition of thromboxane synthase (26). Indeed, it has been reported that peroxyxinitrite not only can inhibit PGH₂ synthase but can also activate PGH₂ synthase (16), which may lead to an accumulation of PGH₂. Therefore, the augmented TP-mediated vasoconstriction observed in OZRs might be a result of PGH₂ accumulation, secondary to increased superoxide anion. Technical limitations did not allow for a direct assessment of TXB₂ or PGH₂ levels within the microvasculature of the spinotrapezius following muscle contraction or AA administration, and the major cellular source(s) for these vasoactive AA metabolites in spinotrapezius muscle in response to the stimuli remains unclear. Alternatively, the impaired functional hyperemia in the present study may involve an upregulation in TP receptor expression. Traupe et al. (29) also demonstrated in obese mice that the enhanced arteriolar vasoconstriction in response to ACh is due, in part, to elevated TP receptor expression. Whether other signaling pathways are involved in this enhanced vasoconstriction should be investigated in future studies.

In the present study, SQ-29548 failed to completely normalize the functional and AA-induced dilations in the OZR (Figs. 2 and 4), suggesting that additional factors such as decreased PGI₂-induced vasodilation may also contribute to the impaired functional hyperemia. Indeed, in the present study, the vasodilatory responses to iloprost were decreased at all concentrations in OZR compared with LZR. These results are consistent with the findings of Frisbee (7, 8) who demonstrated that iloprost-induced arteriolar dilation in isolated gracilis muscle vessels was blunted in 13- to 15-wk-old OZRs. This finding may be the result of decreased expression or sensitivity of IP₃ receptor. Nasrallah and Hébert (23) showed a decreased renal IP₃ receptor mRNA and protein level in response to hyperglycemia. To our knowledge, no studies have been performed to investigate IP₃ receptor expression in this model of metabolic syndrome. Furthermore, we cannot exclude the possibility of a decreased PGI₂ production and/or impaired ATP-sensitive potassium channel function, which may also contribute to the attenuated vasodilatory responses in OZR.

In the present study, OZR exhibited blunted endothelium-dependent vasodilation, whereas the adenine-induced dilation (endothelium-independent) was not different between groups (Fig. 1). The SNP-mediated vasodilation was decreased in OZRs (Fig. 5), implying a decrease in arteriolar sensitivity to NO. Frisbee and Stepp (9) also found a reduced vasodilatory response to SNP in gracilis arteries from 13- to 15-wk-old OZR and suggested that the impaired vasodilatory response is due to an increase in superoxide anion levels. However, SNP-induced vasodilation appears to be dependent on the experiment model and vascular bed. For example, SNP-induced dilation of isolated cerebral arteries was similar between 17-wk-old LZRs and OZRs (25). SNP-induced vasodilation was similar to controls for individuals with type 2 diabetes, although both ACh-induced and functional vasodilation were blunted (14). Previous studies have suggested that NO may not be important in mediating functional hyperemia (2, 27, 28, 31). Therefore, the reduced sensitivity to NO in OZRs most likely does not contribute to the impaired functional hyperemia seen in OZRs. In addition, SQ-29548 treatment had no effect on the vasodilatory response to SNP in both LZRs and OZRs. Future studies are needed to determine the mechanism(s) involved in the reduced arteriolar sensitivity to NO in this model.

In summary, the present study was designed to determine the mechanisms responsible for the impaired functional hyperemia in metabolic syndrome. The functional and AA-induced dilation is significantly attenuated in OZR compared with the LZR. SQ-29548 partially restores the reduced dilation in the OZR but had no effect in LZRs, suggesting the impaired functional dilation in the OZR, at least in part, resulted from an increased TP-mediated constriction. In addition, IP₃ receptor reactivity was reduced in the OZR compared with LZR, which may contribute to the impaired functional hyperemia in these animals. Together, the present studies suggest that the impaired functional hyperemia in OZR results from an increased TP-mediated vasoconstriction and a decreased PGI₂-induced vasodilation. Further studies are needed to determine the mechanisms responsible for the abnormal AA metabolism and/or the altered receptors in this model of metabolic syndrome.

ACKNOWLEDGMENTS

The authors thank Jennifer Harris and Jennifer Dearman for technical help with these experiments.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL-51971 and HL-63958.

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