Attenuated PGI₂ Synthesis in Obese Zucker Rats

Benjamin L. Hodnett, Jennifer A. Dearman, Cory B. Carter, and Robert L. Hester

Department of Physiology and Biophysics
University of Mississippi Medical Center
Jackson, MS 39216

Running head: Obesity and PGI₂ Synthesis.

Keywords: Vasodilation, arachidonic acid, microcirculation and exercise

Address correspondence to:

Robert Hester, Ph.D.
Department of Physiology and Biophysics
University of Mississippi Medical Center
2500 North State Street
Jackson, Mississippi 39216-4505
Phone: (601) 984-1837

Copyright © 2008 by the American Physiological Society.
ABSTRACT

In obesity, skeletal muscle blood flow during exercise (functional hyperemia) is impaired. We have indirectly demonstrated that an altered arachidonic acid metabolism is responsible for the impaired functional vasodilation in the obese Zucker rat (OZR), a model of obesity. In this study we tested the hypothesis that there is an impaired release of PGI2, due to a nitrination of PGI2 synthase (PGIS), which is associated with a decreased prostanoid receptor expression. PGI2, PGE2, and TXA2 release were determined in vitro using ELISA under basal conditions and in response to AA administration (50 μM). PGI2 and TXA2 receptor (IP and TP, respectively) immunofluorescence were determined in dispersed vascular smooth muscle cells (VSMCs). Nitrination of tyrosine residues of the PGI2 synthase enzyme was determined using immunoprecipitation and western blot analysis. Following AA administration, PGI2 and PGE2 release were attenuated in OZR as compared to lean controls (LZR). Basal and AA-induced TXA2 release were not significantly different between groups. IP and TP immunofluorescence were not significantly different between OZR and LZR groups. OZR exhibited elevated nitrination of tyrosine residues of PGIS as compared to LZR. These results suggest that alterations in the PGI2 pathway (attenuated PGI2 synthesis), and not the TXA2 pathway (normal TXA2 synthesis/no change in TP receptor expression), underlie the attenuated functional hyperemia in the OZR.
INTRODUCTION

Obesity is a metabolic disorder associated with insulin resistance, hyperglycemia, dyslipidemia, a pro-inflammatory state, elevations in oxidative stress, and endothelial dysfunction. Obesity itself is a risk factor for cardiovascular diseases including hypertension, coronary artery disease, and stroke, as well as other metabolic disorders such as type II diabetes and metabolic syndrome (1). One treatment option for obesity that has been shown to improve weight control is exercise. However, obesity has been shown to limit the normal increase in muscle blood flow during exercise (functional hyperemia) in both human (20, 27) and animal models (7, 41, 42); thus, limiting the benefit of exercise as an effective treatment option for obese patients.

The exact mechanisms underlying the impaired functional hyperemia in obesity are unclear. One potential cause of the impaired blood flow response is obesity-induced endothelial dysfunction resulting in attenuated release of endothelium-derived relaxing factors, several of which have been shown to regulate arteriolar diameter and muscle blood flow during exercise (34). These endothelium-derived relaxing factors include the prostaglandins, metabolites of arachidonic acid (AA) and the cyclooxygenase pathway (11, 19, 23). Prostacyclin (PGI₂) is the primary prostaglandin released from endothelial cells and is considered to be involved in the functional hyperemic response (32, 33). Exercise has been shown to be associated with an increase in PGI₂ levels, whereas inhibition of prostaglandin synthesis has been shown to markedly attenuate functional hyperemia (28, 38, 39). PGI₂ results in vasodilation through activation of the PGI₂ receptor (IP) (14, 31), and stimulation of the IP receptor using PGI₂ analogs has been shown to result in an attenuated vasodilation in the obese Zucker rat (OZR), both under in
vivo (42) and in vitro (7) experimental conditions. The mechanisms underlying the impaired IP receptor-mediated vasodilation in obesity are unclear and may be due to alterations in downstream signaling and effector mechanisms. However, it is unknown whether PGI2 synthesis itself is impaired in the OZR skeletal muscle vasculature, which would potentially result in diminished stimulation of the IP receptor, and an overall decrease in IP receptor-mediated vasodilation.

Another potential mechanism for the impaired functional hyperemic response in the OZR is an increase in production of the vasoconstrictive metabolite thromboxane A2 (TXA2) which could result in an enhanced TXA2 receptor (TP) stimulation, resulting in an enhanced vasoconstriction that might limit vasodilation in response to other mechanisms. It is unclear to what extent TXA2 production is altered in the OZR, as the TP receptor may also be stimulated by the precursor product of both PGI2 and TXA2, PGH2 (29, 30).

The aim of this study was to test the hypothesis that normal arachidonic acid metabolism is altered in the obese Zucker rat, via a decrease in PGI2 synthesis and an increase in TXA2 production. We also examined whether the synthesis of another prostaglandin, PGE2, was impaired in the obese Zucker rat, in order to assess whether prostaglandin production in general might be impacted. PGE2 has also been shown to be released following exercise in humans (16, 17, 39), though its role in the control of skeletal muscle blood flow in rats, much less in obese models, is largely unknown. In addition to measuring these arachidonic acid metabolites, we determined whether alterations in IP and TP receptor immunofluorescence of isolated vascular smooth muscle cells accompanied the altered arachidonic acid metabolism. Finally, we measured
nitration of tyrosine residues of PGI2 synthase (PGIS), a mechanism by which diminished PGI2 synthesis can occur.

MATERIALS AND METHODS

Animals

The experimental protocols for this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Male lean (LZR; \( n = 17; 349 \pm 6 \) g) and obese (OZR; \( n = 14; 522 \pm 12 \) g) Zucker rats (11 – 14 wk-old; Harlan, Inc., Indianapolis, IN) were used for these experiments. All animals were housed two to three animals per cage at 22°C (12-h light-dark cycle) with ad libitum access to food and water. All animals were anesthetized with sodium pentobarbital (65 mg/kg, ip) prior to surgery. Following tissue removal, animals were euthanized by an overdose of sodium pentobarbital. Death was confirmed by a lack of a heart beat and spontaneous breathing.

Prostanoid Synthesis Assay

Both left and right femoral arteries were removed from lean and obese Zucker rats and placed in a Silastic-coated petri dish containing dissection solution [in mM: 130 NaCl, 4 KCl, 1.2 MgSO4, 4 NaHCO3, 1.8 CaCl2, 10 HEPES, 1.18 KH2PO4, 0.03 EDTA, 6 glucose]. The arteries from LZR (\( n = 10 \)) and OZR (\( n = 7 \)) were cut into equal length (4-mm) segments, placed in wells of a 96 well plate containing a buffer solution [in mM: 118.07 NaCl, 6.17 KCl, 2.55 CaCl2, 25 NaHCO3, 5.5 glucose], and equilibrated in a tissue culture incubator for 1 hour. To determine basal prostanoid release, the arteries were removed, placed in fresh buffer, and maintained for 3 hours at 37 °C (5%
CO₂/balance air). The buffer was removed for analysis and replaced with fresh buffer containing 50 μM AA to determine alterations in stimulated prostanoid release. The arteries were incubated for an additional 3 hours in the incubator, following which the samples were collected and stored at -20 °C until analysis. For each animal, the samples were averaged from both femoral arteries to make one data point. PGI₂ and TXA₂ in the buffer were detected by the presence of their stable metabolites, 6-keto-PGF₁α and TXB₂, respectively using commercially available ELISA kits (Neogen Corporation, Lexington, KY). PGE₂ was detected directly using an ELISA kit (Neogen Corporation). The above chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

**Prostacyclin and Thromboxane Receptor Immunofluorescence**

Arterioles were dissected from the gracilis muscles of lean (n = 3) and obese (n = 3) animals and placed in dissection solution. Vascular smooth muscle cells (VSMCs) were dispersed via enzymatic digestion in HBSS containing 26 U/ml papain and 1 mg/ml ditheoerythritol for 15 min at 37 °C, followed by incubation in HBSS containing 2 U/ml collagenase, 1 mg/ml soybean trypsin inhibitor, and 75 U/ml elastase for 12 min at 37 °C. Following the enzymatic digestion, vessels were rinsed with HBSS and triturated to dissociate VSMCs. Cells were then filtered and examined using a light microscope to check for dispersion. Dissociated VSMCs were fixed in 4% paraformaldehyde, plated on glass slides, and dried for approximately 2 hours at 37 °C. Following drying, samples were rehydrated and washed in PBS and then blocked with 5% normal donkey serum in PBS for 1 hour before addition of primary antibodies. VSMCs were incubated overnight at 4 °C with a mouse monoclonal antibody for α-actin (1:200 dilution; Sigma Chemical
Company) and a polyclonal antibody for one of the receptors: rabbit anti-IP receptor or rabbit anti-TP receptor (1:500 dilution; Cayman Chemical Company, Ann Arbor, MI). Samples were then washed with PBS and incubated with the fluorescent secondary antibodies donkey anti-mouse 488 and donkey anti-rabbit Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hour. Slides were then examined using fluorescence confocal microscopy (Leica Microsystems, Bannockburn, IL) with all α-actin labeled cells from each group used for analysis. IP and TP receptor immunofluorescence were normalized to that of α-actin. Chemicals were purchased from Fisher Scientific (collagenase, HBSS, papain, paraformaldehyde; Pittsburgh, PA), Invitrogen Corporation (soybean trypsin inhibitor; Eugene, OR), and Sigma Chemical Company (ditheoerythritol, elastase).

**Nitration of PGI2 Synthase**

Thoracic aortas were collected from anesthetized lean and obese rats and snap frozen in liquid nitrogen (n = 4 each group). Vessels were homogenized in RIPA buffer containing 1% protease inhibitor cocktail (Sigma Chemical Company). Samples were centrifuged at 14,000 × g for 5 min at 4 °C to remove insoluble debris. The supernatant was collected and sample protein concentrations were quantified using the Bradford method. PGI2 synthase was purified from the cell lysate using a protein G immunoprecipitation kit (Sigma Chemical Company). Briefly, equal volumes of cell lysate were incubated and mixed with rabbit anti-PGIS (1:300 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) in spin columns for 4 hours at 4 °C. Protein-G-agarose beads were added to the cell lysate, incubated, and mixed overnight at 4 °C. The beads
were then washed and the precipitated proteins were collected by elution with 1× Laemelli buffer (Sigma Chemical Company). Equal amounts of protein were then separated using SDS-PAGE (4 – 20% gradient Tris·HCl gels; Jule, Inc., Milford, CT) and transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). Blots were blocked for 1 hour in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) and incubated overnight at 4 °C with rabbit anti-PGIS (1:500 dilution). For immunochemical labeling of PGIS, blots were incubated for 1 hour at room temperature with a fluorescent secondary antibody (goat anti-rabbit IgG; 1:5000 dilution; Rockland Immunocchemicals, Inc., Gilbertsville, PA). Following immunochemical labeling of PGIS, the bands were detected and quantified for total PGIS using an Odyssey infrared imaging system (LI-COR Biosciences). Following PGIS detection, blots were then washed in PBS containing 0.1% Tween 20 and incubated overnight at 4 °C with mouse anti-nitrotyrosine (1:200 dilution; Santa Cruz Biotechnology). For immunochemical labeling of nitrotyrosine, blots were then incubated for 1 hour at room temperature with donkey anti-mouse IgG (1:2000 dilution; Abcam, Inc., Cambridge, MA). Following the second immunochemical labeling of the PGIS bands, blots were rescanned to detect and quantify nitrotyrosine residues. The quantification of the PGIS bands for total PGIS and for nitrated PGIS was performed using densitometric analysis of scanned images using Odyssey 1.1 software (LI-COR Biosciences). The ratio of nitrated PGIS to total PGIS was used as an index of nitration.

Statistics and Data Analysis

Prostanoid release data were analyzed using two-way repeated measures analysis of variance. Student-Newman-Keuls post hoc test was used to compare individual
groups. Immunofluorescence and nitration data were analyzed using t-test. A probability of $P \leq 0.05$ was accepted as statistically significant for all comparisons. All data are presented as means ± SE.

RESULTS

Prostanoid Release

Basal PGI$_2$ release was not significantly different between LZR and OZR (Figure 1). AA administration induced a significant increase in PGI$_2$ production in both groups ($P < 0.001$ for LZR and OZR). However, the stimulated PGI$_2$ release in response to AA was significantly impaired in the OZR as compared to the LZR ($P = 0.049$).

Basal and AA-induced TXA$_2$ release were not significantly different between the two groups (Figure 2). In addition, the difference between the basal and AA-induced levels within a group only reached significance for the LZR ($P = 0.002$).

PGE$_2$ results were similar to those for PGI$_2$ (Figure 3). AA induced significant increases in PGE$_2$ synthesis in both groups ($P < 0.001$ for LZR and OZR). Basal PGE$_2$ release was not significantly different between groups; whereas, AA-induced PGE$_2$ levels were significantly lower in the OZR as compared the LZR ($P = 0.019$).

IP and TP Receptor Immunofluorescence

IP receptor immunofluorescence in VSMCs was similar between the LZR ($n = 3$ animals) as compared with the OZR (Figure 4; $n = 3$ animals). Likewise, TP receptor immunofluorescence in VSMCs was similar between the LZR ($n = 3$ animals) and OZR ($n = 3$ animals) groups (Figure 5).
**Nitration of PGI₂ Synthase**

Following immunoprecipitation of the prostacyclin synthase enzyme from thoracic aorta homogenates of lean and obese animals, western blot analyses were used to detect the prostacyclin synthase enzyme itself and nitrated tyrosine residues. Prostacyclin synthase was detected as two sets of bands, an upper band at ~50 kDa, and a lower band at ~30 kDa. For each band, the ratio of nitrated PGIS to total PGIS was quantified. There was no significant difference in Nitrotyrosine:PGIS of the upper band between LZR and OZR (Fig. 6A). Nitrotyrosine:PGIS of the lower band (Fig. 6B) was significantly elevated in the OZR as compared to the LZR ($P = 0.027$).

**DISCUSSION**

The major findings of this study are that 1) PGI₂ synthesis is impaired in the vasculature of obese Zucker rats; 2) TXA₂ production is not elevated in the OZR; 3) PGE₂ synthesis is also impaired in the OZR; 4) IP receptor immunofluorescence is similar between LZR and OZR vascular smooth muscle cells; 5) TP receptor immunofluorescence is not elevated in OZR VSMCs; and 6) nitration of tyrosine residues of prostacyclin synthase is significantly elevated in the OZR vasculature. These findings support our hypothesis that the impaired functional hyperemia in the obese Zucker rat may be due to alterations in normal arachidonic acid metabolism resulting in impaired PGI₂-mediated vasodilation. This altered AA mechanism appears to be due to alterations in the PGI₂-mediated pathway with no alterations in the TXA₂-mediated pathway. Diminished production of PGI₂ is likely due to increased nitration of tyrosine residues of the PGIS enzyme responsible for PGI₂ synthesis.
The obese Zucker rat, a model of obesity similar to humans, exhibits insulin resistance, hyperglycemia, dyslipidemia, endothelial dysfunction, and increased oxidative stress (9, 25, 41). In addition, this model of obesity exhibits significantly impaired functional hyperemia (7, 41, 42). Several mechanisms are thought to play a role in the altered functional hyperemia seen in the OZR including structural remodeling of the microcirculation (5, 8), alterations in NO- (10) and AA-induced vasodilatory responses mechanisms (6, 42), enhanced α-adrenergic reactivity (4, 35), and enhanced myogenic responses (9). However, the exact mechanism(s) underlying this impaired muscle blood flow during exercise remain unclear.

In human studies, exercise has been shown to increase the release of vasodilatory prostaglandins, i.e. PGI2 and PGE2, suggesting that these factors may play an important role in the control of functional hyperemia (16, 39). Indeed, inhibition of prostaglandin formation has been shown to limit the increase in PGI2 and PGE2 in venous blood normally seen during exercise (39) as well as attenuating functional hyperemia (18, 39). PGI2 is synthesized from PGH2, a precursor prostaglandin and product of arachidonic acid metabolism by cyclooxygenase, via prostacyclin synthase and is the principal prostaglandin synthesized by vascular endothelial cells (32, 33). In instances where PGIS activity is compromised, PGH2 accumulates and is shunted into formation of other prostaglandins as well as the vasoconstrictive product, thromboxane A2 (22, 32, 43). In the same studies measuring the increase in prostaglandin production during exercise (16, 17), thromboxane A2 levels were shown to decrease during exercise. Thus, during exercise, the normal increase in PGI2 release coupled with a decrease in TXA2 synthesis would result in an overall vasodilatory response. Alterations in the normal metabolism of
arachidonic acid could lead to a decrease in the production of the vasodilator PGI2 and an increase in the production of the vasoconstrictor TXA2, thus leading to an overall impairment in vasodilation which would potentially limit functional hyperemia. While previous studies from our lab and others have shown that the OZR exhibits impaired vasodilation in response to the PGI2 analog iloprost, it is unclear whether normal production of PGI2 is altered in this model obesity (7, 42). In the present study, we examined whether alterations in the normal arachidonic acid metabolite profile may be a mechanism underlying the altered vasodilatory responses seen in the OZR.

To determine whether PGI2, PGE2, and TXA2 levels were altered in our model of obesity, we developed a protocol based on a similar one using cerebral arteries (24) in order to quantitate basal prostanoid production as well as stimulated prostanoid production in response to exogenous arachidonic acid administration. Compared with the other products measured (PGE2 and TXA2), PGI2 was the predominant prostanoid released by the femoral arteries (Figure 1). Basal levels of PGI2 were not significantly different between the lean and obese animals; however, following incubation with AA, PGI2 release from femoral arteries of obese animals was significantly attenuated relative to arteries from the lean animals. Interestingly, basal and stimulated TXA2 levels were not elevated in the obese as hypothesized (Figure 2). In fact, only arteries from the lean arteries exhibited a significant increase in TXA2 release following AA administration. PGE2, while quantitatively produced in much lower quantities than PGI2, exhibited a similar attenuation following AA administration in the OZR arteries in comparison with the LZR arteries, as well as no significant difference in basal levels (Figure 3). Taken together these results suggest that AA metabolism is altered in the OZR vasculature with
an impairment in the synthesis pathways of the vasodilatory products PGE$_2$ and PGI$_2$, a predominant regulator of functional hyperemia, and no concomitant increase in the production of the vasoconstrictive product TXA$_2$. The PGE$_2$ results indicate that other prostaglandin synthesis mechanisms may be impaired in our model. While human studies have shown that during acute and prolonged exercise, PGE$_2$ levels increase in the blood (3, 21, 39), the role of this prostaglandin in skeletal muscle blood flow control is unclear. PGE$_2$ has been studied more extensively in vasculature beds such as the renal circulation and has been shown to have four receptor subtypes, EP1 – EP4. In the kidney vasculature, EP2 and EP4 stimulation result in vasodilation, whereas EP1 and EP3 stimulation result in vasoconstriction (37). PGE$_2$ may also play a role in other vasculature beds such as the cerebral circulation. Further work needs to be performed to elucidate the role of PGE$_2$ and the EP receptors in skeletal muscle blood flow control.

Previous findings from our laboratory have suggested that the alterations in arachidonic acid metabolism impair functional vasodilation in the OZR through augmented TP receptor-mediated vasoconstriction and attenuated IP receptor-mediated vasodilation (42). However, it was unclear as to the identity of the AA metabolite responsible for the increase in TP receptor-mediated vasoconstriction, since the TP receptor can be activated by both TXA$_2$ and its precursor PGH$_2$ (29, 30). The lack of a significant difference in basal and AA-stimulated TXA$_2$ production in the OZR supports the theory that the enhanced TP-receptor mediated vasoconstriction in the OZR is due to an accumulation of PGH$_2$, resulting from a decrease in PGI$_2$ synthesis, and not an increase in TXA$_2$ synthesis itself (12, 42). This theory is also supported by studies in other animal models and vascular beds in which enhanced vasoconstriction due to AA
administration has been shown to be attenuated following TP receptor blockade but not following inhibition of TXA₂ synthesis (26, 36, 40).

The finding that the OZR exhibits an attenuated PGI₂ release following AA administration (Figure 1) suggests that in addition to the previously mentioned decrease in sensitivity of the OZR vasculature to IP receptor stimulation, this model of obesity does in fact exhibit impaired PGI₂ synthesis which would further limit vasodilation during exercise. Chronic elevations in oxidative stress in the OZR may be the mechanism by which this apparent decrease in PGI₂ synthesis occurs. Frisbee’s laboratory has previously suggested that the impaired hypoxic vasodilation in the OZR vasculature may be due to increased PGI₂ degradation by superoxide (6). However, the primary mechanism by which elevations in oxidative stress may impair PGI₂ release is likely due to inhibition of PGIS (43). In cultured human endothelial cells, high glucose concentrations have been shown to increase superoxide formation, leading to an increase in peroxynitrite formation. Peroxynitrite has been shown to cause inactivation of the PGI₂ synthase enzyme through nitration of tyrosine residues (2, 45, 46). This inactivation in PGI₂ synthase results in an accumulation of PGH₂, which as previously mentioned, can cause vasoconstriction through activation of the TP receptor. Thus, we investigated whether the attenuated PGI₂ release in our model could be attributed to an increase in nitration of PGIS. We used an immunoprecipitation protocol to isolate PGIS from thoracic aorta homogenates from both lean and obese Zucker rats. Nitration of PGIS was determined by incubating western blots of immunoprecipitated PGIS with an antibody to nitrotyrosine (Figure 6). PGIS migrated as two sets of bands, an upper band near 50 kDa and a lower band near 30 kDa. This banding pattern corresponds to that seen in
atherosclerotic vessels, with the multiple bands possibly resulting from PGIS degradation products (44). The lower band had an increased ratio of nitrated PGIS:total PGIS in the OZR compared with the LZR whereas the upper bands showed lower levels of total nitration that was similar between groups. This result suggests an increased nitration of PGIS in the OZR, which corresponds to their already increased oxidative stress levels. Thus, an increase in nitration of PGIS is a potential mechanism explaining the impaired PGI₂ synthesis found in the in vitro studies.

In addition to measuring PGI₂ and TXA₂ levels in our model of obesity, we examined whether the receptors for these AA metabolites were altered in the OZR. In theory, a downregulation of IP receptors and/or an upregulation of TP receptors could potentially result in an altered functional hyperemic response. In order to determine whether IP and TP receptor expression were altered in the OZR rat microvasculature, we utilized an immunofluorescence technique (15) in which vascular smooth muscle cells from intramuscular gracilis arterioles were isolated and stained with antibodies to either IP or TP receptors. Interestingly, we found that the IP receptor immunofluorescence tended to be lower in the OZR cells compared with those from the LZR (Figure 4); however, this trend did not reach statistical significance. The TP receptor immunofluorescence was similar between the two groups (Figure 5). These results suggest that neither an increase in TP receptor expression nor a downregulation of IP receptors in the OZR are likely to be primary mechanisms involved in the altered vasodilatory response. The TP receptor results are in contrast to those seen in carotid arteries in a diet-induced mouse model, where TP receptor mRNA expression levels are elevated relative to lean animals (36). Although TP receptors do not appear to be
upregulated in our model, an accumulation of PGH₂ due to diminished PGI₂ synthesis could still potentially result in increased TP-mediated vasoconstriction in the OZR. We initially theorized that a decrease in IP receptor levels could be a potential mechanism involved in the altered functional hyperemia of the OZR. A decreased IP receptor expression could potentially explain the diminished IP receptor sensitivity to PGI₂ in the OZR. Coupled with attenuation in normal PGI₂ synthesis, decreased IP receptor expression would result in a diminished stimulus for vasodilation during exercise in the OZR. However, our results at this time are inconclusive as to whether IP receptor alterations play a role in the altered vasodilatory responses seen in the OZR. Further investigation is needed to explore the roles of prostanoid receptor expression in the altered vasodilation in the OZR. Other mechanisms play a role in the attenuated functional hyperemia in this model. We have recently found that impaired vasodilation in the OZR may be partially due to a decreased sensitivity of ATP-sensitive potassium channels (Kₐ₅₆), which are involved in the normal IP receptor-mediated vasodilation (13). Thus, upstream as well as downstream vasodilatory mechanisms responsible for AA-induced vasodilation are likely impaired in the obese Zucker rat.

**PERSPECTIVES AND SIGNIFICANCE**

The results of the present study indicate that a decrease in PGI₂ synthesis in the obese Zucker rat skeletal muscle vasculature may be a mechanism underlying the impaired vasodilation in response to exercise. In addition, the obese Zucker rat exhibits an increase in nitration of tyrosine residues of PGI₂ synthase, a mechanism which has been shown to decrease PGI₂ production. Furthermore, the OZR exhibited no elevation in
TXA$_2$ production and no upregulation of the TP receptor. Thus, the altered arachidonic acid metabolism underlying the impaired functional hyperemic response seen in the OZR is most likely due solely to alterations in the PGI$_2$ portion of the pathway, with little to no compounding impairment of the TXA$_2$ response. The enhanced TP-receptor mediated vasoconstriction evident in the OZR is likely due to accumulation of PGH$_2$ resulting from attenuation of normal PGI$_2$ synthesis, and this metabolite’s ability to stimulate the TP receptor. Although many questions remain concerning the role of altered arachidonic metabolism in the attenuated functional hyperemia in obesity, it is anticipated that the present findings may play a role in the future development of treatment protocols that improve muscle blood flow during exercise in obese patients, as well as adding to the base of knowledge in this area of physiological research.
Acknowledgements

This work was supported by an AHA Predoctoral Fellowship, NIH HL51971, and a UMC Intramural Grant.
REFERENCES


16. **Karamouzis M, Karamouzis I, Vamvakoudis E, Ampatzidis G, Christoulas K, Angelopoulou N, Mandroukas K.** The response of muscle interstitial prostaglandin E\textsubscript{2}(PGE\textsubscript{2}), prostacyclin I\textsubscript{2}(PGI\textsubscript{2}) and thromboxane A\textsubscript{2}(TXA\textsubscript{2}) levels during incremental


FIGURE LEGENDS

Figure 1. Basal and arachidonic acid-induced PGI2 release from femoral arteries of lean \((n = 10)\) and obese \((n = 7)\) Zucker rats. Values are means ± SE. * Significant difference between basal and arachidonic acid-induced PGI2 release. # Significant difference between lean and obese arachidonic acid-induced PGI2 release.

Figure 2. Basal and arachidonic acid-induced TXA2 release from femoral arteries of lean \((n = 10)\) and obese \((n = 7)\) Zucker rats. Values are means ± SE. * Significant difference between basal and arachidonic acid-induced TXA2 release.

Figure 3. Basal and arachidonic acid-induced PGE2 release from femoral arteries of lean \((n = 10)\) and obese \((n = 7)\) Zucker rats. Values are means ± SE. * Significant difference between basal and arachidonic acid-induced PGE2 release. # Significant difference between lean and obese arachidonic acid-induced PGE2 release.

Figure 4. IP receptor immunofluorescence from dispersed VSMCs of gracilis arterioles from lean and obese Zucker rats. Cells were from 3 lean and 3 obese animals. Values are means ± SE. Immunofluorescence levels were normalized to \(\alpha\)-actin. There was no significant difference between groups.

Figure 5. TP receptor immunofluorescence from dispersed VSMCs of gracilis arterioles from lean and obese Zucker rats. Cells were from 3 lean and 3 obese animals. Values
are means ± SE. Immunofluorescence levels were normalized to α-actin. There was no significant difference between groups.

Figure 6. Tyrosine nitration of prostacyclin synthase of thoracic aorta homogenates from lean (n = 4) and obese (n = 4) Zucker rats. (A) Upper bands (~50 kDa) and (B) lower bands (~30 kDa) were quantified as the ratio of nitrated prostacyclin synthase to total prostacyclin synthase. Values are means ± SE. * Significant difference between lean and obese groups.
Figure 1

6-keto-PGF1α Concentration (ng/ml)

Lean  |  Obese

- Basal
- Arachidonic Acid (50 μM)

*  
#
Figure 2

The graph shows the TXB2 concentration (pg/ml) in Lean and Obese individuals under Basal and Arachidonic Acid (50 μM) conditions. The concentration is significantly higher in the Arachidonic Acid condition compared to the Basal condition, as indicated by the asterisk (*) on the graph.
Figure 3

PGE2 Concentration (pg/ml)

Basal

Arachidonic Acid (50 μM)

Lean

Obese

* * #
Figure 4

IP Receptor

α-actin

Lean Obese

IP Receptor / SM α-actin

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8

Lean Obese

Bar graph showing the comparison of IP Receptor / SM α-actin levels between Lean and Obese groups.
Figure 5

TP Receptor

α-actin

Lean Obese

TP Receptor / SMα-actin

0.0 0.1 0.2 0.3 0.4 0.5

Lean Obese

20.00 μm
Figure 6

A

Nitrotyrosine

PGIS

Lean

Obese

B

Nitrotyrosine

PGIS

Lean

Obese

Upper Band Nitrotyrosine / PGIS

Lean

Obese

Lower Band Nitrotyrosine / PGIS

Lean

Obese

*