Blunted temporal activity of microvascular perfusion heterogeneity in metabolic syndrome: a new attractor for peripheral vascular disease?

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Submitted 26 October 2012; accepted in final form 12 December 2012

Butcher JT, Goodwill AG, Stanley SC, Frisbee JC. Blunted temporal activity of microvascular perfusion heterogeneity in metabolic syndrome: a new attractor for peripheral vascular disease? Am J Physiol Heart Circ Physiol 304: H547–H558, 2013. First published December 21, 2012; doi:10.1152/ajpheart.00805.2012.—A key clinical outcome for peripheral vascular disease (PVD) in patients is a progressive decay in skeletal muscle performance and its ability to resist fatigue with elevated metabolic demand. We have demonstrated that PVD in obese Zucker rats (OZR) is partially due to increased perfusion distribution heterogeneity at successive microvascular bifurcations within skeletal muscle. As this increased heterogeneity ($\gamma$) is longitudinally present in the network, its cumulative impact is a more heterogeneous distribution of perfusion between terminal arterioles than normal, causing greater regional tissue ischemia. To minimize this negative outcome, a likely compensatory mechanism against an increased $\gamma$ should be an increased temporal switching at arteriolar bifurcations to minimize downstream perfusion deficits. Using in situ cremaster muscle, we determined that temporal activity (the cumulative sum of absolute differences between successive values of $\gamma$, taken every 20 s) was lower in OZR than in control animals, and this difference was present in both proximal (1A–2A) and distal (3A–4A) arteriolar bifurcations. Although adrenergic receptor blockade (phentolamine) improved temporal activity in 1A–2A arteriolar bifurcations in OZR, this was without impact in the distal microcirculation, where only interventions against oxidant stress (Tempol) and thromboxane A$_2$ activity (SQ-29548) were effective. Analysis of the attractor for $\gamma$ indicated that it was not only elevated in OZR but also exhibited severe reductions in range, suggesting that the ability of the microcirculation to respond to any challenge is highly restricted and may represent the major contributor to the manifestation of poor muscle performance at this age in OZR.

rodent models of obesity; microcirculation; skeletal muscle blood flow regulation; models of peripheral vascular disease; blood flow heterogeneity; vascular dysfunction

WITH ONGOING STUDY, THERE is increasing appreciation that the transition from healthy physiology to disease states represents a change in the overall system of control from an existing normal structure (46). It is also generally unclear whether this transition represents a failure of the healthy system or evolving compensations to attempt to optimize the most critical biological outcomes despite progression of a challenged environment (38, 57). For this reason, a detailed understanding of which major contributing processes to an outcome are altered with pathology and the extent for which these are compensated is critical for developing appropriate therapeutic interventions. Failure to elucidate both the key sites of impairment and the compensatory mechanisms, as well as how these impact functional outcomes, has resulted in poorly targeted interventional measures.

Although the existing literature contains extensive prior interrogation into the effects of atherosclerotic peripheral vascular disease on perfusion and performance outcomes in multiple model systems as well as the identification of many putative mechanistic contributors to plaque/lesion development (1, 9, 20, 27, 30, 39), investigation into the impact of nonatherosclerotic peripheral vascular disease (PVD) has received a much more limited investment. Although underrepresented to date, this is an exceedingly important area of investigation, since a growing body of evidence suggests that an increasing number of human subjects are afflicted with the symptomology of PVD in the absence of overt plaque/lesion development (12, 19, 31, 44, 45).

Based on its origin in a dysfunctional leptin receptor gene, destroying the satiety reflex, the obese Zucker rat (OZR) experiences a chronic hyperphagia, developing severe obesity, a progressive worsening of glycemic control and plasma lipid profiles and, ultimately, the development of moderate hypertension (32, 37). The importance of the OZR model for studying nonatherosclerotic PVD is considerable, since the genesis of the pathological state lies within the leptin resistance-induced hyperphagia, which is found in the human population (53, 56). Furthermore, the severity of the elevated risk factors for a negative cardiovascular outcome has repeatedly been demonstrated to be comparable with that determined in afflicted patients, and these animals do not develop atherosclerotic lesions.

A recent study has provided clear and compelling evidence that impairments to skeletal muscle performance that are associated with development of nonatherosclerotic PVD in OZR are not adequately predicted by basic indexes such as the mechanical responses of resistance arterioles to stimuli or by bulk blood flow responses to skeletal muscle (25). Rather, we have recently demonstrated that a defining characteristic of nonatherosclerotic PVD in OZR skeletal muscle is an increasingly asymmetric blood flow distribution at successive microvascular bifurcations, resulting in pronounced heterogeneity of perfusion across the distal arterioles within the network (24, 59). Although this increased spatial heterogeneity of perfusion distribution was improved by interventions against reactive oxygen stress, the actions of thromboxane, and altered function within adrenergic vasoconstriction (24, 59), the question of the system compensation for this increased perfusion heterogeneity in OZR is entirely unknown. Conceptually, a likely compensation for the increased spatial heterogeneity of perfusion in the microcirculation of OZR could be via increased temporal switching at arteriolar bifurcations. Because the spatial hetero-
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Table 1. Baseline characteristics of ~17-wk-old LZR and OZR used in the present study

<table>
<thead>
<tr>
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<th>LZR</th>
<th>OZR</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>Mass, g</td>
<td>357 ± 9</td>
<td>676 ± 10*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>104 ± 4</td>
<td>135 ± 6*</td>
</tr>
<tr>
<td>[Glucose]plasma, mg/dl</td>
<td>106 ± 9</td>
<td>188 ± 12*</td>
</tr>
<tr>
<td>[Insulin]plasma, ng/ml</td>
<td>1.7 ± 0.2</td>
<td>8.8 ± 1.3*</td>
</tr>
<tr>
<td>[Nitrotyrosine]plasma, ng/ml</td>
<td>17 ± 4</td>
<td>53 ± 7*</td>
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Values are means ± SE. *P < 0.05 vs. lean Zucker rats (LZR). OZR, obese Zucker rats; MAP, mean arterial pressure.

Monitoring γ throughout the observation period and analyzing those resulting data used three initial approaches. The first of these was simply to determine the means ± SE of the individual measurements throughout the 5-min observation window, providing the mean value for γ. However, this is clearly not sufficiently informative in terms of understanding the temporal behavior for γ at a bifurcation. The second approach was to determine the cumulative change in γ from each of the 15 successive measurements: Σ(15)y(t+1) − y(t))y(t), where γ represents the perfusion distribution at any given bifurcation and t represents the measurement time from 1 (initial) to 15 (final). Although these data can provide us with evidence of broader changes in γ with longer durations, it was apparent that a more sensitive marker is needed to provide sufficient insight into temporal switching at an arteriolar bifurcation. As such, the final approach is to summate the absolute differences in γ at one time point to the next to provide a superior index of total activity: Σ(14)y(t+1) − y(t))y(t).

This final index (summatiing absolute difference in γ) will provide insight into the total temporal switching at any arteriolar bifurcation within the data collection window.

To determine the effectiveness of any intervention on improving the behavior of γ over the data collection window in OZR, the values for the γ (cumulative, absolute differences) that were collected for arteriolar bifurcations in LZR under control conditions (A) were set as the overall control value. As such, the impact of pathology (development of the metabolic syndrome) is determined from the data collected in OZR under control conditions (B) and is the absolute difference between these two values: z = ABS(A − B). The extent to which any intervention in OZR can improve normal behavior is given by the value of γ (cumulative, absolute) following the intervention (C) and is represented by y = ABS(B − C). Given this, the percent effectiveness of any intervention in restoring the normal behavior of γ at a bifurcation in OZR is provided by z = (y/x) × 100.

An attractor is generally defined as the set of conditions toward which a given variable evolves over time. To a significant extent, this can be considered as the representation of the behavior of a dynamic system over a period of time, and this can be extremely useful in understanding the limits of a system (i.e., what behaviors are either not possible, not generally attainable, or are extremely unlikely) and the effects on system behavior (either beneficial or detrimental) subsequent to an imposed intervention. The most informative manner in presenting an attractor is as an iterated map, where the current state of the parameter under study is plotted at a given moment and then its change in position replotted at the next time interval. This process is iterated repeatedly, to the conclusion of the data set, and the shape and location of the attractor on the coordinate system becomes evident. This approach also facilitates comparisons between experimental conditions and the impact these have on the location and shape of the attractor.

All data throughout the manuscript are presented as means ± SE. Statistically significant differences in measured and calculated parameters were determined using a one sample t-test (differences from 0), Student’s t-test, or ANOVA with Student-Newman-Keuls post hoc test used as needed. In all cases, P < 0.05 was taken to reflect statistical significance.

RESULTS

Table 1 presents the baseline characteristics of animals used in the present study. By ~17 wk of age, OZR demonstrated striking obesity and had a significantly higher mass than LZR. Furthermore, OZR exhibited statistically significant elevation in mean arterial pressure, and both plasma insulin and nitrotyrosine concentrations. Data describing the dimension and perfusion characteristics of the cremasteric arteriolar bifurcation segments (described above) under the conditions of the present study are summarized in Table 2. These data clearly demonstrate general differences between arteriolar diameter and perfusion characteristics that are distributed longitudinally throughout the cremasteric microcirculation under the conditions of the present study.

Figure 2 presents representative data describing the distribution of the magnitude of γ and its temporal behavior across

Table 2. Arteriolar perfusion in LZR and OZR

<table>
<thead>
<tr>
<th></th>
<th>LZR</th>
<th>OZR</th>
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<tbody>
<tr>
<td>ID</td>
<td>95 ± 4</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>Vbrb</td>
<td>56 ± 6</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>Q</td>
<td>248 ± 12</td>
<td>135 ± 15*</td>
</tr>
<tr>
<td>2A</td>
<td>70 ± 5</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>Vbrb</td>
<td>50 ± 4</td>
<td>34 ± 6</td>
</tr>
<tr>
<td>Q</td>
<td>120 ± 12</td>
<td>60 ± 7*</td>
</tr>
<tr>
<td>3A</td>
<td>54 ± 5</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>Vbrb</td>
<td>35 ± 6</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>Q</td>
<td>50 ± 5</td>
<td>25 ± 4*</td>
</tr>
<tr>
<td>4A</td>
<td>37 ± 5</td>
<td>32 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE and presented for arteriolar inner diameter (ID; in μm), centerline erythrocyte velocity (V(brb); in mm/s), and volume perfusion within the arteriole (Q; in nl/s). *P < 0.05 vs. LZR; †P < 0.05 vs. OZR. P, treatment with phentolamine; T, treatment with Tempol; SQ, treatment with SQ-29548, with combinations representing treatment with multiple agents.
1A-2A (Fig. 2, A and B, respectively) and 3A-4A (Fig. 2, C and D, respectively) arteriolar divisions. As suggested by these data, in addition to a shift in the frequency distribution of \( \gamma \) between LZR and OZR at both microvascular divisions, the changes in \( \gamma \) (measured every 20 s over the course of 5 min) were less extensive in both proximal and distal bifurcations of the resting cremaster muscle microcirculation of OZR compared with LZR.

**Proximal resistance arterioles.** The summarized results of the average magnitude of \( \gamma \) at 1A-2A arteriolar bifurcations over the observation period are presented in Fig. 3. In LZR (Fig. 3A), \( \gamma \) was ~0.51 under control conditions and was not

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Fig. 2. Frequency distribution and representative sample of the temporal changes in \( \gamma \) in 1A-2A (A and B, respectively) and 3A-4A (C and D, respectively) arteriolar bifurcations in lean Zucker rats (LZR) and obese Zucker rats (OZR) under control conditions. For data in B and D, \( \gamma \) is determined every 20 s throughout a 5-min collection window.

![AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00805.2012 • www.ajpheart.org](http://ajpheart.physiology.org/)

Fig. 3. The average \( \gamma \) in 1A-2A arteriolar bifurcations of LZR (A) and OZR (B) over the 5-min collection period. Data (presented as means ± SE) are presented for LZR and OZR under control conditions and in OZR after treatment of the cremaster muscle with phentolamine (Phent), Tempol (Tem), SQ-29548, or combinations of these agents. * \( P < 0.05 \) vs. control in that strain.
altered by acute pharmacological intervention. In contrast, average $\gamma$ over the 5-min period in OZR (Fig. 3B) was much higher, ~0.58 under control conditions. Although interventions containing phentolamine (regardless of the presence of any other substance) reduced average $\gamma$ to levels determined in LZR, treatment against either the increased TxA2 production (SQ-29548) or the elevated vascular oxidant stress (Tempol) in OZR were not effective, resulting in minimal changes to $\gamma$.

Data describing the changes in $\gamma$ in 1A-2A bifurcations between LZR and OZR over the 5-min observation window are summarized in Fig. 4. When presented as the cumulative change in $\gamma$, neither LZR (Fig. 4A) nor OZR (Fig. 4B) exhibited a difference between strains or a clear pattern in terms of the cumulative change in $\gamma$ within a strain. However, when the cumulative changes in $\gamma$ are presented as the sum of the absolute changes, striking differences are evident. Although a clear reduction in the cumulative change in $\gamma$ (absolute) was evidence in OZR (Fig. 4D) as compared with LZR (Fig. 4C), blockade of adrenoreceptors (alone or with any other pharmacological intervention) in 1A-2A bifurcations in LZR reduced activity at the bifurcation. In contrast, treatment of elevated oxidant stress with Tempol or blockade of TxA2 with SQ-29548 has no discernible impact. In 1A-2A bifurcations from OZR, treatment with phentolamine (either alone or with Tempol and/or SQ-29548) significantly increased the cumulative change in $\gamma$ (absolute) over the data collection window (Fig. 4D). Similarly to LZR, treatment with Tempol and/or SQ-29548 in the absence of phentolamine did not have a significant impact.

**Distal resistance arterioles.** Comparable with data presented for proximal resistance arterioles, the average $\gamma$ for 3A-4A arteriolar bifurcations over the 5-min period was ~0.3 in LZR under control conditions (Fig. 5A), and this was elevated in OZR (Fig. 5B). However, although treatment interventions were without effect in LZR, application of either Tempol or SQ-29548 in OZR significantly reduced mean $\gamma$ toward 0.5. In contrast with the results from more proximal resistance arterioles, $\gamma$ was largely unaffected by phentolamine treatment and interventions targeted at rectifying endothelial dysfunction were required to cause a significant restoration of $\gamma$.

Figure 6 presents the cumulative changes in $\gamma$ over the 5-min period in 3A-4A arteriolar bifurcations from LZR (Fig. 6, A and C) and OZR (Fig. 6, B and D). When the changes in $\gamma$ are presented as simple accumulation, there were no significant differences between LZR (Fig. 6A) and OZR (Fig. 6B) or within a strain as a result of any intervention. However, when the changes in $\gamma$ are summed as absolute differences, clear differences were identified. In 3A-4A arteriolar bifurcations of LZR (Fig. 6C), acute treatment with phentolamine reduced the accumulated changes to $\gamma$, whereas the presence of Tempol and/or SQ-29548 resulted in minimal total changes or marginal change (when applied in combination with each other or with

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**Fig. 4.** Data describing the cumulative changes in $\gamma$ over the 5-min collection period in 1A-2A arteriolar bifurcations in LZR and OZR. Data (presented as means ± SE) are presented for LZR and OZR under control conditions and in OZR after treatment of the cremaster muscle with phentolamine (Phent), Tempol (Tem), SQ-29548, or combinations of these agents. Cumulative changes in $\gamma$ are summated either as differences between successive time points (A and B) or as the absolute differences between successive time points (C and D). *$P < 0.05$ vs. control in that strain; †$P < 0.05$ vs. LZR control.
In contrast, application of Tempol and/or SQ-29548 significantly improved the cumulative changes to $\gamma$ in 3A-4A arteriolar bifurcations of OZR, whereas the effects of phentolamine application were muted (Fig. 6D).

Effects of adenosine. To simulate the impact of elevated metabolic demand on the temporal stability of $\gamma$ throughout the cremaster muscle microcirculation, increasing concentrations of adenosine were added to the preparation superfusate. Although application of adenosine had minimal impact on average $\gamma$ in LZR, it reduced average $\gamma$ in OZR, at both 1A-2A (Fig. 7A) and 3A-4A (Fig. 7C) bifurcations to levels that were very similar to that determined in LZR, with the greatest impact being observed with the highest level of adenosine (Fig. 7). Adenosine did not impact the cumulative change in $\gamma$ over the 5-min period in either strain, at either bifurcation location (data not shown). However, when the changes in $\gamma$ are summated as

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)

Fig. 5. The average $\gamma$ in 3A-4A arteriolar bifurcations of LZR (A) and OZR (B) over the 5-min collection period. Data (presented as means ± SE) are presented for LZR and OZR under control conditions and in OZR after treatment of the cremaster muscle with phentolamine (Phent), Tempol (Tem), SQ-29548, or combinations of these agents. * $P < 0.05$ vs. control in that strain.

![Graph A](image5)

![Graph B](image6)

![Graph C](image7)

![Graph D](image8)

Fig. 6. Data describing the cumulative changes in $\gamma$ over the 5-min collection period in 3A-4A arteriolar bifurcations in LZR and OZR. Data (presented as means ± SE) are presented for LZR and OZR under control conditions and in OZR after treatment of the cremaster muscle with phentolamine (Phent), Tempol (Tem), SQ-29548, or combinations of these agents. Cumulative changes in $\gamma$ are summated either as differences between successive time points (A and B) or as the absolute differences between successive time points (C and D). * $P < 0.05$ vs. control in that strain; † $P < 0.05$ vs. LZR control.
absolutes, application of increasing concentrations of adenosine reduced temporal activity in LZR at both 1A-2A (Fig. 7B) and 3A-4A (Fig. 7D) bifurcations, although it had minimal impact on activity in the bifurcations of OZR (Fig. 7, B and D).

**Effectiveness of interventions in restoring normal behavior.** Figure 8 summarizes the impact of the interventions on restoring normal temporal activity at 1A-2A (Fig. 8A) and 3A-4A (Fig. 8B) arteriolar bifurcations in OZR (where the activity at those bifurcation levels in untreated LZR is defined as control/normal). As shown in Fig. 8A, although there is a significant recovery in function with treatment with the adrenoreceptor antagonist alone, combined therapy of phentolamine with the
agents targeted at improving endothelial dysfunction resulted in a dramatic improvement in the temporal behavior of $\gamma$ at the proximal arteriolar bifurcations of OZR. Conversely, the reverse pattern was evident for distal arteriolar bifurcations of OZR, as treatment with SQ-29548 and/or Tempol resulted in significant improvements to the temporal activity of $\gamma$, whereas the combined application with phentolamine resulted in a further marginal improvement to this outcome.

**Establishment of a new attractor for PVD.** Figure 9 presents the attractor for $\gamma$ at 1A-2A arteriolar bifurcations in cremaster muscle of LZR and OZR (control conditions). As show in Fig. 9A, the attractor for changes in $\gamma$ in OZR was shifted from that in LZR, exhibiting a higher $\gamma$, and a smaller range over which $\gamma$ can move. Treatment with phentolamine (Fig. 9B) was effective in restoring the magnitude of the attractor in OZR compared with that in LZR, although range remained restricted. In contrast, treatment of the cremasteric network with Tempol and/or SQ-29548 was of minimal benefit in restoring the attractor in OZR to that in LZR (Fig. 9C). However, combined treatment with phentolamine and TEMPOL/SQ-29548 almost completely restored the magnitude and the shape of the attractor in OZR to that in LZR. This situation was reversed in distal arteriolar bifurcations of OZR and LZR (Fig. 10), as the shift in the attractor for OZR versus LZR under control conditions (Fig. 10A) was much more strongly impacted by treatment with Tempol and/or SQ-29548 (Fig. 10C) than with phentolamine (Fig. 10B); although combined treatment with phentolamine and Tempol/SQ-29548 still resulted in near complete restoration of the attractor shape and magnitude determined in LZR (Fig. 10D).

The effects of increasing adenosine concentration on the attractor in arteriolar bifurcations of OZR are summarized in Fig. 11. In both proximal (Fig. 11A) and distal (Fig. 11B) arteriolar bifurcations, treatment with high concentrations of adenosine ($10^{-3}$ M) restored the attractor magnitude, but not the full extent of the shape. Lower concentrations of adenosine ($10^{-7}$ or $10^{-5}$ M) were without discernible effect (data not shown). The temporal behavior of $\gamma$ suggested that, with the highest concentrations of adenosine, there was a less heterogeneous distribution of perfusion at bifurcations, but also that the activity of $\gamma$ over time remained reduced.

**DISCUSSION**

It has long been understood that the development of non-atherosclerotic PVD, with its implicit failure of appropriate matching of blood perfusion to meet requisite metabolic demand, is associated with a severely compromised ability of the skeletal muscle to maintain performance (i.e., developed tension levels) (19, 44). However, with the use of the OZR model of the metabolic syndrome, it has been clearly demonstrated that blood-perfused skeletal muscle rates of fatigue are increased as compared with LZR and that this compromised performance is not simply a function of global ischemia (25). Rather, treatment with an adrenoreceptor antagonist and a substantial restoration of functional hyperemia, muscle fatigue rates, and oxygen uptake (VO$_2$) remained compromised in OZR as compared with LZR. It was only after combined treatment with both the adrenoreceptor antagonist and interventions ameliorating endothelial dysfunction (the antioxidant Tempol and/or the PGH$_2$/TxA$_2$ receptor antagonist SQ-29548)
that muscle performance and VO₂ were improved. These results strongly suggest a spatial divergence with regard to dysfunction wherein altered adrenoreceptor function primarily impacts bulk perfusion resistance while altered endothelial function primarily impairs perfusion:demand matching in the distal microvasculature (25). We have interrogated this spatial divergence in blood flow control in skeletal muscle of OZR and have determined that a defining characteristic of PVD in this model is an increasingly heterogeneous distribution of blood flow at successive bifurcations within the arteriolar network (defined by the proportionality parameter: γ). The deviation of γ from 0.5 (homogeneous distribution) is not only strongly predictive of poor muscle performance, it was determined to be longitudinally consistent down the arteriolar network, and was demonstrated to lead to severe increases in blood flow distribution at the precapillary level in OZR as compared with LZR (24, 59).

The elevated γ at arteriolar bifurcations and increased spatial heterogeneity of perfusion distribution represent a deviation from the normal system behavior identified in microvascular networks of LZR. However, as with the majority of biological systems, compensatory mechanisms that can minimize the impact of the increased spatial heterogeneity in perfusion should be present. Logically, the most likely compensatory mechanism would be an increased temporal switching at the individual bifurcations (an increased rate of change with regard to which daughter represents the high or low flow branch, or at least in the severity of the change in γ), which would serve to minimize the extent to which signals associated with any developed ischemic signals accumulate within a low flow branch. Although the nature of these signals is not fully clear at this time, with potential contributors stemming from the buildup of parenchymal tissue metabolic signals (10, 13, 17, 49), substances released from increasingly deoxygenated
erythrocytes as they pass through the microcirculation (22, 29, 50, 51), or undefined signals that are propagated along myocyte and/or endothelial communication pathways (3, 7, 21, 55), the general principle appears to be valid regardless of the stimuli involved.

The data presented in this study refute our general hypothesis, as the accumulated shifts in \( \gamma \) with time at both 1A-2A (Fig. 3 of 4) and 3A-4A (Fig. 5 of 6) arteriolar bifurcations in resting skeletal muscle of OZR were significant reduced as compared with levels determined in LZR. This is a particularly striking observation, as it clearly suggests that not only does a change in temporal switching at arteriolar bifurcations not serve as a compensatory mechanism for the increased \( \gamma \) at arteriolar bifurcations in OZR, the reduction in temporal switching actually entrenches the negative outcomes for perfusion distribution associated with the increased \( \gamma \). The results from subsequent interventions demonstrated that this blunting of temporal activity in arteriolar bifurcations of OZR could be ameliorated. In proximal resistance arterioles, treatment of the resting cremaster muscle with phentolamine, to remove alterations to the distribution of adrenergic constriction throughout the network, significantly increased the cumulative \( \gamma \), an observation that was not identified in the distal microcirculation, where adrenoreceptor blockade was not associated with a significant change in \( \gamma \). Conversely, interventions targeted at correcting endothelial dysfunction (Tempol and/or SQ-29548) had minimal impact on the temporal activity of perfusion distribution at proximal arterioles but significantly improved this response in more distal 3A-4A bifurcations (Fig. 8). In all cases, combined treatments improved \( \gamma \) to levels that were very similar to that determined for LZR under control conditions. It is particularly compelling that these interventions and their sites of greatest impact parallel results in our previous studies with regard to correcting the spatial heterogeneity of \( \gamma \) throughout the microcirculation (24) and improving muscle fatigue resistance and \( \text{VO}_2 \) (24). Specifically, although treatment with either phentolamine or Tempol/SQ-29548 alone improved select or localized indexes of vascular function (with no beneficial impact on muscle performance), only combined treatment with phentolamine and Tempol/SQ-29548 resulted in a widely distributed and significant improvement to muscle perfusion, \( \text{VO}_2 \), and performance (25). Taken together, this series of observations suggest that, with a combined intervention designed to remove alterations to adrenergic constriction and endothelial dysfunction in the OZR model of metabolic syndrome, blood flow hemodynamics at successive arteriolar bifurcations is improved to a condition which helps to restore the processes of mass transport and exchange in the microcirculation to a level that allows for a significant improvement to muscle performance. However, the extent to which this is manifested as a change to the total surface area available for exchange in the muscle or an improvement to erythrocyte distribution within the terminal arterioles and capillary networks remains to be elucidated.

One of the benefits derived from the increased effort of acquiring temporal data is that it can allow for the determination of an attractor. In essence, an attractor is the set to which a variable will adhere over time (36, 58). Even following perturbation as a result of an imposed challenge, the variable will change its behavior over time to return to its attractor if possible (36, 58). In the present experiments, the attractor for both proximal (1A-2A) and distal (3A-4A) arteriolar bifurcations in LZR exhibited a wide range of states around a central region with \( \gamma \) approximating 0.5. This was dramatically different in both proximal (Fig. 9) and distal (Fig. 10) arteriolar bifurcations of OZR, where the central region of the attractor was shifted to \(-0.58\), with a narrowed range of achieved values. Beyond being an intriguing scientific curiosity, this shift in the attractor has critical implications for blood flow control in OZR. The change in both magnitude and range of the attractor with the development of pathology in OZR indicates that the ability of arterioles and arteriolar bifurcations to respond to imposed challenges (e.g., changes in metabolic demand) will be severely compromised as the potential for perfusion redistribution will be constrained to a more limited set of attainable conditions. Essentially, the development of nonatherosclerotic PVD in OZR is defined by a loss of flexibility in the control systems contributing to the regulation of perfusion distribution in skeletal muscle, thus severely compromising the ability to effectively adapt to changing conditions. This is a particularly striking outcome and it may represent the more functionally integrated extension of a similar process identified by Tigno et al. (54) in diabetic monkeys, where the randomness of arterial vasomotion was found to be dampened with disease progression. Taken together, it is not simply that we have dysfunction and can identify it in a series of reduced preparations. It is that indexes of dysfunction in simplified preparations, when integrated, produce an outcome wherein the pathology is partially defined by an impaired ability of the system to respond to stressors.

Despite numerous attempts, we were unable to acquire a sufficiently robust data set that outlined the impact of muscle contraction on \( \gamma \) in the cremaster muscle microcirculation. However, as a simulator of this condition, we used elevated concentrations of adenosine in the preparation superfusate and determined the resulting effects on \( \gamma \). The results clearly indicate that superfusion with low (10\(^{-7}\) M) or moderate (10\(^{-3}\) M) levels of adenosine had a limited impact on the temporal behavior of \( \gamma \) or the resulting attractor in arteriolar bifurcations of OZR, whereas superfusion with high concentrations of adenosine (10\(^{-3}\) M) nearly restored \( \gamma \) to levels determined in arteriolar bifurcations in LZR, but did not restore the range of the attractor (Fig. 11). These observations strongly suggest that the arteriolar networks in OZR may be resistant responding to elevated metabolic demand, until a threshold is reached. Once a sufficient metabolic intensity (or build-up of the appropriate signal) has been attained, the system will respond, although to a constrained extent (i.e., magnitude of \( \gamma \) is restored, but range remains blunted). As we have demonstrated previously (24), this high concentration of adenosine results in a maximal relaxation of in situ skeletal muscle arterioles in OZR, meaning that the entire vascular network should be without any significant source of tone. This would mean that any changes in \( \gamma \) would reflect only the impact of the architecture and geometric arrangement of the network, as well as the intrinsic physical processes associated with blood flow and erythrocyte distribution in vascular networks, as has been previously studied by Cokelet (14, 15) and Kiani et al. (33), among others (23, 28). As presented in Fig. 11, the attractor for \( \gamma \) under these conditions is very similar between LZR and OZR in terms of magnitude and shape, but with a restricted range.
Given the nature of the data in the present study, attention should be given to the impact of the method of anesthesia on outcomes and data interpretation. Numerous previous studies have demonstrated that both inhalational volatile and injectable general anesthetics can impact endothelial function and vaso-motion (16, 41, 48), as well as baroreflex sensitivity and the sympathetic control of vascular tone (48). As such, either option presents a challenge for data interpretation in the present study, especially given the complications associated with altered baroreflex control (40, 47), sympathetic output (11, 47), and endothelial function (25) that has been demonstrated in the obese Zucker rat. Although for this research, we have elected to use a barbiturate anesthetic (owing to the time between exposure and data collection, and to avoid the impact on vasomotion), these issues are that become extremely complicated and further investigation of the impact of anesthetic regimen on microvascular outcomes in models of increased PVD risk may be warranted.

Obviously, the ultimate outcome for microvascular function, regardless of condition, is the ability of the networks to adequately support mass transport and exchange under both resting and challenged conditions. If we assume that the attractors identified for LZR under control conditions are representative of health, for the purposes of the present discussion it may be useful to consider this as optimized (i.e., these attractors describe microvascular networks functioning optimally). With this assumption, the attractors for OZR would represent the resultant state of the microcirculation under conditions of the metabolic syndrome. As such, it is important to estimate how these changes to perfusion distribution would impact indices such as tissue oxygenation or microvascular hematocrit. Although these measurements are outside of the scope of the current study, previous research efforts can provide some potential insight. In particular, the work of Pries et al. (42, 43) has identified a network Fahraeus effect that can provide some potential insight. In particular, the work of Pries et al. (42, 43) has identified a network Fahraeus effect that can represent a significant contributor to the ultimate reduction in microvascular hematocrit at successive bifurcations owing to a disproportionate distribution of flow (and therefore, erythrocytes) at arteriolar bifurcations. Under control conditions, those authors suggested that this alone could cause a reduction in microvascular hematocrit approaching 20%, with direct implications for tissue oxygenation (8, 52). As the results from the present study clearly indicate that γ is increased in the arteriolar networks of OZR (vs. control), it would be logical to speculate that this would result in an exacerbation of the network Fahraeus effect, which, when taken in combination with other contributors to hematocrit reduction [e.g., the vessel Fahraeus effect (15, 43) and other hemorheological parameters (5, 23, 34)], would result in a further reduction of microvascular hematocrit within the networks. Although specific interventions such as adrenoceptor blockade and inhibiting the actions of TXA2 could provide some amelioration of this impairment, based on alterations to the shape of the attractors, only a combined interventional strategy would be sufficient to cause an improvement to arteriolar network function that would be sufficient to result in alterations to mass transport and exchange that were large enough to improve muscle fatigue resistance (25). Further investigation into the impact of these cumulative perfusion abnormalities in the skeletal muscle microcirculation of OZR could provide us with a far more complete understanding of how nonatherosclerotic PVD is manifest in this increasingly relevant animal model of the metabolic syndrome.

ACKNOWLEDGMENTS

We thank Milinda James from the Department of Physiology and Pharmacology at West Virginia University for expert technical assistance and the Translational Research Facility in the Center for Cardiovascular and Respiratory Sciences at the West Virginia University Health Sciences Center for support.

GRANTS

This study was supported by grants from the National Institutes of Health (NIH DK-R01-64668, T32-HL-90610, and RR-2865AR) and the American Heart Association (AHA EIA 0740129N).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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