High-intensity interval and endurance training are associated with divergent skeletal muscle adaptations in a rodent model of hypertension

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Holloway TM, Bloemberg D, da Silva ML, Quadrilatero J, Spriet LL. High-intensity interval and endurance training are associated with divergent skeletal muscle adaptations in a rodent model of hypertension. Am J Physiol Regul Integr Comp Physiol 308: R927–R934, 2015. First published April 8, 2015; doi:10.1152/ajpregu.00048.2015.—Skeletal muscle is extremely adaptable to a variety of metabolic challenges, as both traditional moderate-intensity endurance (ET) and high-intensity interval training (HIIT) increases oxidative potential in a coordinated manner. Although these responses have been clearly demonstrated in healthy individuals, it remains to be determined whether both produce similar responses in the context of hypertension, one of the most prevalent and costly diseases worldwide. Therefore, in the current study, we used the Dahl sodium-sensitive rat, a model of hypertension, to determine the molecular responses to 4 wk of either ET or HIIT in the red (RG) and white gastrocnemius (WG) muscles. In the RG, both ET and HIIT increased the content of electron transport chain proteins and increased succinate dehydrogenase (SDH) content in type I fibers. Although both intensities of exercise shifted fiber type in RG (increased IIA, decreased IIX), only HIIT was associated with a reduction in endothelial nitric oxide synthase and an increase in HIF-1α proteins. In the WG, both ET and HIIT increased markers of the electron transport chain; however, HIIT decreased SDH content in a fiber-specific manner. ET increased type IIA, decreased IIB fibers, and increased capillarization, while, in contrast, HIIT increased the percentage of IIB fibers, decreased capillary-to-fiber ratios, decreased endothelial nitric oxide synthase, and increased hypoxia inducible factor-1α (HIF-1α) protein. Altogether, these data show that unlike in healthy animals, ET and HIIT have divergent effects in the skeletal muscle of hypertensive rats. This suggests ET may be optimal at improving the oxidative capacity of skeletal muscle in animals with hypertension.

HYPERTENSION REMAINS ONE OF THE greatest contributors to heart-related mortality (27) and the development of heart failure (HF) worldwide (1, 27). While directly affected by hypertension, HF remains a complex disorder that manifests with systolic dysfunction, HF is also associated with a derangement in whole blood pressure elicit detrimental adaptations. However, lower-intensity exercise in a model of hypertension has also been shown to be insufficient to induce mitochondrial biogenesis (10), highlighting the necessity for additional research, examining the effectiveness of exercise training protocols that involve different fiber recruitment patterns in the presence of hypertension.

Therefore, it remains to be determined whether both ET and HIIT increase the oxidative potential of skeletal muscle in the presence of high blood pressure. The present study aimed to determine whether ET and HIIT improved the oxidative potential of skeletal muscle in Dahl sodium-sensitive (Dahl/SS) rats. We hypothesized that both ET and HIIT would increase skeletal muscle is implicated in the prevalence of exercise intolerance in HF patients; however, they are amenable to exercise training (20, 42). Therefore, elucidating which exercise intensities improve the oxidative capacity of skeletal muscle is essential for clinical care of individuals with hypertension, as they are at elevated risk for the development of HF and exercise intolerance.

Skeletal muscle has a remarkable ability to adapt to exercise training, such that a single bout of exercise is sufficient to induce the activation of transcription factors involved in the regulation of the metabolic profile (37). Mitochondrial biogenesis (involving both proliferation and alterations in mitochondria) results from the cumulative effects of repeated transient upregulation of the mRNAs of factors involved in mitochondrial and metabolic adaptations (37). Coordinated responses, such as increases in mitochondrial content, elevated maximal activities of oxidative enzyme, and higher expression of plasma membrane transporters, all facilitate a higher oxidative potential of skeletal muscle (11, 23, 24, 38, 39, 53). In healthy individuals, these adaptations appear to occur independently of the intensity of training; as both high-intensity interval training (HIIT) and moderate-intensity endurance training (ET) induce these responses (7, 8, 18, 19, 21, 35, 36, 50, 52). These data suggest that HIIT may provide an equally effective and time-efficient alternative to ET.

While the molecular adaptations to exercise within skeletal muscle appear to be independent of exercise intensity in healthy individuals, this remains to be determined in the presence of hypertension or compromised cardiac performance. The clinical relevance of HIIT remains debatable, especially within the context of hypertension and HF, as unlike ET, HIIT does not uniformly increase stroke volume (21, 31). In rodent models of hypertension, extreme or exhaustive exercise has also been associated with negative adaptations in the heart (2, 16, 26, 46). As a result, it has been suggested that an “intensity threshold” exists in pathological conditions, such that higher intensities of exercise in the presence of high blood pressure elicit detrimental adaptations. However, low-intensity exercise in a model of hypertension has also been shown to be insufficient to induce mitochondrial biogenesis (10), highlighting the necessity for additional research, examining the effectiveness of exercise training protocols that involve different fiber recruitment patterns in the presence of hypertension.

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markers of whole muscle mitochondrial content, shift fiber composition toward a slower, more fatigue-resistant fiber type, and improve the skeletal muscle capillary-to-fiber ratio similarly in Dahl/SS rats, providing support for the use of HIIT in the presence of hypertension.

METHODS

Animals and experimental design. The Dahl/SS rat develops hypertension, HF, and an increased risk of mortality on a high-sodium diet within 8 wk, and it follows the most prevalent known human progression of hypertension-induced HF (41, 44). Given the rapid progression toward HF, these animals are ideal for elucidating the optimal intensity of exercise as a lifestyle intervention to improve the oxidative potential of skeletal muscle.

We examined the effects of ET and HIIT in male Dahl/SS rats compared with sedentary (SED) animals. Dahl/SS rats (8 wk of age; n = 18) and were fed high-sodium chow to induce the hypertensive phenotype. The diet containing 8% sodium chloride was purchased through Research Diets (New Brunswick, NJ). The animals were randomly assigned to three experimental conditions: SED (n = 6), classical ET (n = 6), or HIIT (n = 6). The high-sodium diets and exercise interventions commenced at the same time point (e.g. week 1). Animals were housed one per cage in a temperature-regulated room on a 12:12-h light-dark cycle with water available ad libitum. This study was approved by the University of Guelph Animal Care Committee and conforms to the guide for the care and use of laboratory animals published by the U.S. National Institutes of Health.

Treadmill exercise. All rats were familiarized with a rodent treadmill (Columbus Instruments, Columbus, OH) on at least three occasions (10 m/min, 0% grade, 10–15 min) before randomization. Briefly, at the same time as the initiation of the HS diet, both the ET and HIIT animals trained 5 days/wk for 4 wk at a progressively more challenging intensity. The ET animals trained beginning at 10–15 m/min at 0% grade and progressed to 20 m/min at 10% grade for 45 min at week 4. HIIT animals began by alternating between active rest (2 min at 10 m/min, 0% grade) and high intensity (10 m/min, 10% grade) for 30 min and progressed to intervals of 2-min active rest and high intensity (10 m/min, 10% grade and 20 m/min, 15% grade) for 45 min at week 4. The average work intensity (Joules/min) (34) was 148.4 ± 5.6 for SED, 151.6 ± 5.1 for ET, and 151.6 ± 4.4 for HIIT. These intensities were selected based on the following criteria: (i) a 15% grade on a rodent treadmill was considered to constitute a challenging intensity. The ET animals trained beginning at 10–15 m/min at 0% grade and progressed to 20 m/min at 10% grade for 45 min at week 4. HIIT animals began by alternating between active rest (2 min at 10 m/min, 0% grade) and high intensity (10 m/min, 10% grade) for 30 min and progressed to intervals of 2-min active rest and high intensity (10 m/min, 10% grade and 20 m/min, 15% grade) for 45 min at week 4. The average work intensity (Joules/min) (34) was 148.4 ± 5.6 for SED, 151.6 ± 5.1 for ET, and 151.6 ± 4.4 for HIIT. These intensities were selected based on the following criteria: (i) a 15% grade on a rodent treadmill was considered to constitute a challenging intensity.

Statistical analysis. Data, with the exception of fiber type, are represented as a percentage of SED. Fiber types are represented as a percentage of total fibers within SED, ET, and HIIT. Values were analyzed for significance compared with SED using a paired t-test with the α-value set to P < 0.05.

RESULTS

Systolic and diastolic blood pressure. We first aimed to ensure that the high-sodium diet elicited hypertension. All animals displayed both systolic (SED: 189 ± 3, ET: 189 ± 4, HIIT: 192 ± 1 mmHg) and diastolic (SED: 149 ± 1, ET: 151 ± 2, HIIT: 150 ± 2 mmHg) hypertension, vs. Dahl/SS rats fed low-sodium chow (44). However, 4 wk of ET or HIIT did not alter (P > 0.05) blood pressure.
Markers of mitochondrial content. We next aimed to characterize the effects of ET and HIIT on the oxidative capacity of the skeletal muscle in this model of hypertension. ET and HIIT increased \( P < 0.05; \sim 25\% \) the protein content of various markers of the electron transport chain (i.e., OXPHOS) in the RG (Fig. 1, A and B). In the WG, ET, and HIIT increased \( P < 0.05 \) the protein content of the OXPHOS subunits (Fig. 1, C and D). In addition, while ET and HIIT did not alter the activity of CS in the RG (Fig. 2A), both exercise intensities increased SDH content in type I muscle fibers specifically, which represent \sim 45\% of the fibers within the RG (Table 1). However, in contrast to the RG, both ET and HIIT decreased \( P < 0.05 \) CS in the WG (Fig. 2B) by \sim 25\%, while SDH content was decreased in IIB fibers following HIIT (Table 1).

Fiber type composition. Given the variable responses in the oxidative capacity of muscle to exercise training in the RG and WG, we next examined MHC content within each muscle type. Within the RG, both ET and HIIT resulted in an increase in the percentage of type IIA and a decrease in IIX fibers, compared with SED, however, only ET resulted in a twofold increase in IIAx intermediate fibers (Fig. 3A and Table 1). Within the WG, ET, and HIIT had divergent effects on fiber type. Specifically, ET resulted in a reduction IIB and IIXB fibers and a three-fold increase in the percentage of IIX fibers (Fig. 3B and Table 1). In contrast, HIIT resulted in a reduction in IIXB and an increase in IIB fibers (Fig. 3B and Table 1).

Capillarization and angiogenic factors. Given the divergent effects of ET and HIIT on the oxidative capacity and fiber-type profile of skeletal muscle, we next characterized the effect of these training regimes on capillary density. The capillary-to-fiber ratio was decreased in the RG following both ET and HIIT (Fig. 4, A and B). However, this was only associated with a reduction in eNOS (Fig. 5B), and increased HIF-1α protein.
Table 1. Fiber type, CSA, and SDH in SED, ET, and HIIT rats

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>SED</th>
<th>ET</th>
<th>HIIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber Count</td>
<td>Type I</td>
<td>Type IIA</td>
<td>Type IIA</td>
</tr>
<tr>
<td>%Population</td>
<td>359 ± 38</td>
<td>45.0 ± 3.5</td>
<td>28.1 ± 1.4</td>
</tr>
<tr>
<td>CSA, μm</td>
<td>539 ± 40</td>
<td>3759.0 ± 95.9</td>
<td>2464.2 ± 169</td>
</tr>
<tr>
<td>SDH, AU</td>
<td>20.7 ± 1</td>
<td>35.5 ± 1.1</td>
<td>24.3 ± 0.3*</td>
</tr>
</tbody>
</table>

| Red Gastrocnemis | |
|------------------|------------------|--------------|
| CSA, μm | 524 ± 69 | 3672.4 ± 207 | 2316.4 ± 87.3 |
| SDH, AU | 24.3 ± 0.3* | 37.1 ± 0.4 | 27.5 ± 0.4 |

| White Gastrocnemis | |
|-------------------|------------------|--------------|
| CSA, μm | 566 ± 6.4 | 3955.4 ± 174.9 | 2696.5 ± 149* |
| SDH, AU | 25.1 ± 0.8* | 36.9 ± 0.7 | 26.2 ± 1.1 |

Values are expressed as means ± SE; n = 6 per group. SED, sedentary; ET, endurance training; HIIT, high-intensity interval training; CSA, cross-sectional area; SDH, succinate dehydrogenase. *P < 0.05 vs. SED.

(Fig. 5C) following HIIT, suggesting hypoxia occurred only in the HIIT animals. In addition, in the WG, ET increased the capillary-to-fiber ratio (Fig. 4, C and D), while HIIT decreased the capillary-to-fiber ratio (Fig. 4, C and D) and increased the protein content of HIF-1α (Fig. 5G); once again suggesting HIIT induced hypoxia. ET and HIIT did not alter the protein contents of VEGF or its receptor, VEGFR2, in either the RG or WG (Fig. 5, D, E, H, I).
DISCUSSION

In the current study, we provide evidence that in a model of hypertension, both ET and HIIT increased markers of OXPHOS protein content and SDH content in type I fibers and induced a transition toward a slower, fatigue-resistant fiber type in the RG. However, HIIT induced a reduction in eNOS protein and an increase in HIF-1α protein. In the WG, ET and HIIT both increased markers of the electron transport chain. However, although ET shifted the WG toward a fatigue-resistant fiber type, and promoted angiogenesis, HIIT shifted the WG toward a fast, fatigable fiber type, decreased capillary-to-fiber ratios, decreased eNOS, and increased HIF-1α protein. Altogether, these data demonstrated that unlike in healthy animals, ET and HIIT have divergent effects in the skeletal muscle of hypertensive rats and suggest that ET may be optimal at improving the overall oxidative capacity of skeletal muscle in animals with hypertension.

Markers of mitochondrial content. Skeletal muscle mitochondrial proliferation is a well-known adaptation to exercise training (23, 24). In the current study, both ET and HIIT increased the protein content of various electron transport chain subunits in the RG, suggesting both intensities of exercise increase the oxidative potential of skeletal muscle. These adaptations were more robust in type I fibers, as only type I fibers in the RG displayed an increase in SDH activity. These data support previous work in healthy individuals, which has shown that both ET and HIIT increase various markers of mitochondrial content and have a similar response in type I and II fibers (47, 49). It was surprising that neither ET nor HIIT increased CS activity in the current study in the RG. However, since SDH only increased in type I fibers, which represent ∼45% of the fibers in the RG, it is likely that small CS adaptations within type I fibers would be undetectable at the mixed homogenate level. Altogether, ET and HIIT displayed similar mitochondrial responses in the RG.

Both ET and HIIT decreased CS activity and SDH content in type IIX and IIB fibers in the WG. This finding was unexpected; however, these data clearly suggest that typical exercise adaptations are compromised in a model of disease. Nevertheless, previous work has demonstrated inconsistencies in the response of CS activity after exercise training in spontaneously hypertensive rats (22, 32, 45). While both ET and HIIT increased markers of the electron transport chain, these adaptations suggest the absence of a coordinated increase in the oxidative capacity within the WG following both ET and HIIT in a model of hypertension.

Fiber-type composition in response to exercise. Fiber-type transitions occur under conditions of overloading, such as exercise training (39, 40). In general, endurance exercise training promotes a slower, fatigue-resistant fiber type (38). This transition follows a sequential order from type IIB to IIX to IIA to I, along with the appearance of hybrid fibers. In the current study, both ET and HIIT promoted a significantly higher percentage of type IIA fibers and fewer IIX. However, only ET promoted a shift toward IIAX hybrid fiber. In contrast, within the WG, ET induced an increase in IIX fibers, while HIIT increased type IIB fibers. These fiber transitions suggest that in a hypertensive model, ET promotes a slower, fatigue-resistant fiber type, while HIIT promotes a faster, fatigable fiber type. Although it is well known that recruitment plays a role in exercise-related fiber transitions (38), HIIT resulted in adaptations in WG electron transport chain proteins, suggesting widespread recruitment was likely. Although the etiology of the divergent responses in fiber-type transitions remains unclear, it is possible that during exercise, the HIIT animals are unable to maintain the cardiac output required by the exercis-
skeletal muscle. Regardless of the mechanism, the current data suggest that ET is the more appropriate form of training in the presence of hypertension, a finding that may also extend to other pathological situations associated with impaired skeletal muscle oxidative capacity.

Capillarization, eNOS, and HIF-1α content. A key determinant of the increased matching of metabolic demand and oxygen (O₂) delivery to the skeletal muscle posttraining is due to proliferation in capillary beds within the trained muscle (11). During exercise training, active muscle experiences repetitive oxygen stress (a fall in intracellular partial pressure of O₂), such that exercise training promotes angiogenesis (43). The resultant increase in capillary beds allows for a more coordinated metabolic response to exercise, such that blood flow is more closely matched to tissues with increased metabolic demand. Surprisingly, within the RG, both ET and HIIT animals experienced a reduction in capillary density compared with SED animals. This decrease in capillarization was only accompanied by reduced eNOS and an increase in HIF-1α protein in the HIIT animals. These data suggest hypoxia may have occurred, or may have been more prominent, in the HIIT animals. HIF-1α suppresses mitochondrial biogenesis and is considered the primary transcriptional factor responsible for adaptations to hypoxic stimuli, such as repetitive exercise training (48, 57). Chronic exercise training in healthy subjects results in lower HIF-1α protein content (28). Our data demonstrate that ET increased the capillary-to-fiber ratio in the WG, while HIIT decreased the capillary-to-fiber ratio, stimulating an increase in HIF-1α protein content. Once again, this suggests that HIIT potentially induced a greater hypoxic stimuli in the contracting muscle. In contrast, previous reports in healthy individuals have shown that capillary density and eNOS content are increased as a result of both ET and HIIT (13, 35). However, in a rodent model of Type 2 diabetes, ET preferentially increased the capillarization of oxidative muscles supporting the current interpretation that ET and HIIT have divergent effects in pathological conditions (30).

In conclusion, our data provide evidence that in hypertensive Dahl/SS rats, ET increased the percentage of IIX fibers and expression of oxidative proteins in RG and preferentially increased IIX fibers and capillary density in WG. Conversely, while HIIT increased the percentage of IIX fibers and oxidative proteins, it was associated with a reduction in eNOS content in RG, an increase in IIB fibers, and reduced capillarization in the WG.

Perspectives and Significance

Altogether, the current data suggest that in animals with hypertension, ET may represent the optimal stimulus for coordinated adaptations resulting in enhanced skeletal muscle oxidative capacity. The mechanism by which HIIT elicited...
negative responses in skeletal muscle remains unknown, but may be explained by a local redox imbalance, as the larger fluctuations in metabolites during HIIT (e.g., IMP) would be anticipated to increase ROS to a greater degree. ROS is required for many physiological signaling processes and stimulates increases in the expression of HIF-1α (33), as observed following HIIT. However, disproportionate levels of ROS decreases the downstream activation of VEGFR2 (5), impacting VEGF-mediated angiogenesis, and potentially explaining the reduction in capillarization following HIIT. While this mechanism remains speculative, exercise training in the presence of existing oxidative stress (e.g., aging) is not associated with the beneficial effects of exercise (54, 55). These data suggest that exercise-mediated increases in ROS can be detrimental in situations with oxidative stress/diminished antioxidant defenses. Therefore, future studies should investigate the effects of ET and HIIT in hypertensive humans, and the associated changes in the oxidative state of the skeletal muscle.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: T.M.H. and L.L.S. conception and design of research; T.M.H., D.B., and M.L.d.S. performed experiments; T.M.H. and D.B. analyzed data; T.M.H., D.B., J.Q., and L.L.S. interpreted results of experiments; T.M.H. prepared figures; T.M.H. drafted manuscript; T.M.H., D.B., J.Q., and L.L.S. approved final version of manuscript.

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