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Type II Diabetes Accentuates Diaphragm Blood Flow Increases During Submaximal Exercise in the Rat

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Abstract

We investigated the effect of type 2 diabetes mellitus (T2DM) on respiratory muscle blood flow (BF) during exercise. Using the Goto-Kakizaki (GK) rat model of T2DM, we hypothesized that diaphragm, intercostal and transverse abdominis BFs (radiolabeled microspheres) would be higher in male GK rats ($n=10$) compared to healthy male Wistar controls (CON; $n=8$) during submaximal exercise (20 m/min, 10% grade). Blood glucose was significantly higher in GK (246 ± 29 mg/dL) compared to CON (103 ± 4 mg/dL; $P<0.01$). Respiratory muscle BFs were not different at rest ($P>0.50$). From rest to submaximal exercise, respiratory muscle BFs increased in both groups to all muscles ($P<0.01$). During submaximal exercise GK rats had higher diaphragm BFs (GK: 189 ± 13 ; CON: 138 ± 14 mL/min/100g, $P<0.01$), and vascular conductance (GK: 1.4 ± 0.1 ; CON: 1.0 ± 0.1 mL/min/mmHg/100g; $P<0.01$) compared to CON. There were no differences in intercostal or transverse abdominis BF or VC during exercise ($P>0.15$). These findings suggest that submaximal exercise requires a higher diaphragm BF and VC in T2DM compared to healthy counterparts.

Keywords

Goto-Kakizaki diabetic rat; respiratory muscles; intercostals; vascular conductance; exercise tolerance

1. Introduction

Approximately 30 million Americans have diabetes mellitus, of which ~90–95% have Type 2 diabetes mellitus (T2DM), and an additional ~84 million Americans are considered prediabetic (Benjamin et al., 2019). Patients suffering from T2DM are characterized by

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compromised exercise tolerance (Gurdal et al., 2015; Poitras et al., 2018) and a lack of adherence to physical activity guidelines (Morrato et al., 2007; Thomas et al., 2004). This is unfortunate given that physical activity/exercise has protective effects on glycemia and insulin sensitivity (Colberg et al., 2010; Kearney and Thyfault, 2016; Sigal et al., 2004). Thus, understanding the mechanistic underpinnings of exercise intolerance is crucial to improve clinical outcomes and quality of life for this patient population.

Patients with T2DM exhibit pulmonary system alterations that likely contribute to reduced exercise capacity and would be expected to increase the work of the respiratory muscles and thus blood flow (BF) requirement to these muscles during exercise. For example, compared to healthy controls, T2DM patients exhibit a reduced vital capacity and total lung volume (Anandhalakshmi et al., 2013; Fontaine-Delaruelle et al., 2016), loss of elastic properties of the lung and respiratory muscle strength (Anandhalakshmi et al., 2013; Correa et al., 2011; Fuso et al., 2012; Zineldin et al., 2015), thickening of the alveolar epithelial and endothelial capillary basal lamina (Foster et al., 2010; Weynand et al., 1999), and impaired lung diffusion capacity at rest and during submaximal exercise (Anandhalakshmi et al., 2013; Chance et al., 2008; Saler et al., 2009). During exercise, T2DM patients, compared to healthy adults, have an exaggerated ventilatory response relative to CO₂ production (*i.e.* higher $\dot{V}_E/\dot{V}_{CO_2}$) to maintain alveolar ventilation (Roberts et al., 2018; Tantucci et al., 1996; Vukomanovic et al., 2019), which is independent of common comorbidities such as coronary artery disease and heart failure (Gurdal et al., 2015). Together, these structural and functional pulmonary system derangements may culminate in greater respiratory muscle work and therefore demand a higher respiratory muscle BF during exercise in T2DM than healthy counterparts; however, this has not been investigated.

We have used the Goto-Kakizaki (GK) rat model of T2DM to determine the effect of T2DM on capillary hemodynamics (Padilla et al., 2006), microvascular oxygen pressures (Padilla et al., 2007), and on hindlimb muscle weight and muscle BF at rest and during submaximal running (Copp et al., 2010). Most relevant to the current investigation, we found ~20% lower hindlimb muscle weight normalized to body weight ratios in GK rats (Copp et al., 2010), which is consistent with multiple reports of lower lean body mass in human T2DM patients compared to healthy adults (Kiely et al., 2014; Kim et al., 2014; Park et al., 2006). Further, we found that during submaximal treadmill exercise, BF was not different between GK and Wistar control rats for the majority of the hindlimb muscles, but was significantly higher in GK rats for several primarily glycolytic muscles and muscle regions (Copp et al., 2010). The lower hindlimb muscle weight normalized to body weight ratio and higher BF to primarily glycolytic muscle may mechanistically contribute to a higher respiratory muscle BF in T2DM subjects compared to healthy adults, although this has yet to be investigated. Specifically, an increased recruitment of glycolytic muscle fibers may produce a higher $\dot{V}_{CO_2}$ and contribute to the greater ventilatory response during exercise reported in T2DM patients (Roberts et al., 2018; Tantucci et al., 1996; Vukomanovic et al., 2019). Thus, there is a pressing need to investigate the effect of T2DM on respiratory muscle BF during exercise.

The purpose of this investigation was to determine the effects of T2DM on respiratory muscle BF and vascular conductance (VC) at rest and during exercise. Specifically, we tested the hypothesis that diaphragm, intercostal, and transverse abdominis muscle BFs and

VCs would be higher during submaximal treadmill running (20 m/min, 10% incline) in the GK rat model of T2DM compared to CON rats. The same absolute submaximal treadmill running speed was selected as the exercise paradigm because individuals with T2DM and healthy individuals often perform daily tasks, such as walking and climbing stairs, that are equivalent in absolute terms.

2. Methods and Materials

2.1 Experimental animals and ethical approval

The present investigation focuses on the effect of T2DM on respiratory muscle BF at rest and during submaximal exercise in the same animals used in a previously published dataset (Copp et al., 2010). The T2DM model selected for this investigation was the male GK rat (Taconic Farm, Germantown, NY; 6–8 mo old; $n = 10$). Healthy male Wistar controls (CON) rats (Taconic Farm, Germantown, NY; 6–8 mo old; $n = 8$) served as age-matched controls. Healthy Wistar rats are appropriate controls because they derive from the same original strain as the GK rat. The GK model is a hyperglycemic, hyperinsulinemic nonobese rat strain developed by breeding glucose-intolerant Wistar rats selectively (*i.e.* ~ 5 generations; Goto and Kakizaki, 1981). These GK rats require no specially formulated diet (Goto et al., 1976), and develop hyperglycemia at ~12 weeks of age (Lang et al., 2009). Thus, the GK rats in the present investigation were hyperglycemic for ~10–18 weeks, which is equivalent to ~7–12 years in humans given the differences in lifespans.

All rats were maintained in a controlled environment with a fixed 12:12-h light-dark cycle and room temperature maintained at ~22°C. All rats were provided standard rodent chow and water ad libitum. All experimental conditions and surgical procedures were approved by the Kansas State University Institutional Animal Care and Use Committee and are consistent with the guidelines mandated by the National Institutes of Health.

2.2 Surgical instrumentation

All rats were familiarized with running on a motor-driven treadmill according to the Resource Book for the Design of Animal Exercise Protocols published by the American Physiological Society (Kregel et al., 2006). During the period of familiarization (5–8 runs performed over 2–3 wk), rats exercised for 5–10 min/day at a speed of 20 m/min and 10% grade. After it was established that all rats were proficient runners, each animal was fasted overnight before acute instrumentation for the final experimental protocol. On the day of the experiment, each rat was weighed and anesthetized with 5% isoflurane. Under aseptic conditions, a shallow incision was made on the midline of the anterior portion of the neck to allow access to the carotid artery. The right carotid artery was exteriorized and cannulated with a 2-Fr-catheter-tipped pressure micromanometer (model TC-510, Millar Instruments). While heart rate (HR) and the arterial pressure waveform was being monitored, the pressure transducer was advanced into the left ventricle (LV) in a retrograde fashion and LV end-diastolic pressure (LVEDP) and the derivative of the pressure wave form (LV dP/dt) were measured and recorded. After LVEDP and LV dP/dt measurements were completed, the micromanometer was removed and replaced with a catheter (PE-10 connected to PE-50) that was placed in the ascending aorta. A second catheter (PE-10 connected to PE-50) was placed

in the caudal (tail) artery, as previously described (Musch and Terrell, 1992). Both catheters were tunneled subcutaneously to the dorsal aspect of the cervical region and exteriorized through a puncture wound in the skin. After the closure of incisions, anesthesia was terminated, and the animal was given 2 h to recover. This period of recovery was selected to avoid changes in tissue glycogen content as a result of the catheterization (Moore et al., 1988) that may impact running performance negatively. Additionally, the recovery time for all animals was equivalent to account for the possibility of the confounding effects of the anesthesia regimen (Lawson et al., 2001), although, importantly, it has been demonstrated that a window of 1–6 h of recovery after anesthesia elicits stable cardiac and circulatory dynamics, regional blood flow, arterial blood gases, and acid-base status in the awake unrestrained rat (Flaim et al., 1984; Poole et al., 2020).

2.3 Final experimental protocol

Subsequent to the recovery period, and before the final experimental protocol was initiated, a small arterial blood sample (~50 µl) was taken for blood glucose concentration (Accu-Check Advantage, Roche Diagnostics, Indianapolis, IN) as previously described (Padilla et al., 2007). Each rat was then placed on the treadmill, and after a period of stabilization (3 h after instrumentation), the tail artery catheter was connected to a 1-ml plastic syringe that was connected to a Harvard infusion/withdrawal pump (model 907, South Natick, MA). The carotid artery catheter was connected to a pressure transducer set at the same level as the rat for continuous measurement of arterial blood pressure and HR. Exercise was initiated, and the speed of the treadmill was increased progressively during the next 30 s to a speed of 20 m/min (10% grade). The rat then exercised steadily for another 3 min. After 3.5 min of total exercise time, blood withdrawal from the tail artery catheter was initiated at a rate of 0.25 ml/min. Simultaneously, arterial blood pressure and HR were measured via the carotid artery catheter. After 4 min of total exercise time, the carotid artery catheter was disconnected from the pressure transducer and ~5–6 × 10⁵ microspheres with a 15-µm diameter (isotopes used were ⁴⁶Sc, ⁸⁵Sr, ¹¹³Sn, or ¹⁴¹Ce, in random order; Perkin Elmer Life and Analytical Sciences, Boston, MA) were injected into the aortic arch to determine regional blood flow. It has been demonstrated previously in awake rats that multiple injections of 15-µm diameter microspheres with less than a total of 1.4 × 10⁶ microspheres do not produce any hemodynamic disturbances including changes in cardiac output, blood pressure, heart rate and total peripheral resistance (Stanek et al. (1983). Approximately 30 s after the injection, blood withdrawal from the tail artery catheter was stopped and exercise was terminated. After a 60-min recovery period, hemodynamic parameters were measured as the rat sat quietly on the treadmill. A second microsphere injection was then performed with the same procedures as described above to determine resting respiratory muscle blood flow. This sampling strategy minimizes the potential for blood loss to affect the exercise response and facilitates “resting” measurements that do not reflect the pre-exercise anticipatory response (Armstrong et al., 1989).

2.4 Determination of BF and VC

Following the completion of the exercise protocols and resting measurements, rats were anesthetized and euthanized with an overdose of sodium pentobarbital (100 mg/kg body wt.) via the right carotid artery catheter. A pneumothorax was performed and placement of

each catheter was verified by anatomic dissection. The lungs were excised and weighed. The left ventricle (LV) and right ventricle (RV) were excised and then the RV was separated from the LV and septum, and both tissues weighed. The diaphragm, intercostals, and transversus abdominis of each rat were then dissected out. The tissues were blotted, weighed, and placed immediately into counting vials. LV with the septum attached, RV, lung and diaphragm were divided by the body weight and are reported as mg/g. The radioactivity of each tissue was determined with a gamma scintillation counter (model 5230, Auto Gamma Spectrometer, Packard, Downers Grove, IL). The radioactivity of the tissues was then analyzed by computer, taking into account the crosstalk fraction between the different isotopes. Absolute muscle BF was then calculated by the reference sample method (Ishise et al., 1980) and expressed in milliliters per min per 100 g of tissue. VC was then calculated by normalizing BFs to mean arterial pressure (MAP) measured at the time of the microsphere infusion and expressed as mL/min/mmHg/100 g. Delta () respiratory muscle BFs (*i.e.* diaphragm BF, Intercostal BF, and Transverse abdominis BF) were calculated as the difference between BF during submaximal exercise and the BF at rest, whereas respiratory muscle VCs (*i.e.* diaphragm VC, intercostal VC, and transverse abdominis VC) were calculated as the difference between VC during submaximal exercise and the VC at rest.

2.5 Statistical analysis.

Values are reported as mean $\pm$ standard error (SE). All statistical analyses were performed using GraphPad Prism Version 8.2. Unpaired Student's *t*-tests were used to compare diaphragm and body weight, blood glucose, LVEDP, LV dP/dt, along with the LV, RV, lungs and diaphragm normalized to body mass (organ weight/body weight ratio). MAP, HR as well as BFs and VCs were compared within (rest vs. exercise) and between groups (GK vs. CON) using two-way mixed factorial analysis of analysis (ANOVA) and Tukey *post-hoc* tests when appropriate. The diaphragm, intercostal, and transversus abdominis BF and VC from rest to exercise were compared between groups via unpaired student's *t*-tests. Simple linear regressions were used to determine if blood glucose correlated with diaphragm, intercostal, and/or transverse abdominis BF during submaximal exercise, if the diaphragm BF correlated with diaphragm weight and diaphragm weight normalized to body weight, and if diaphragm BF correlated with lung weight normalized to body weight. An outlier was detected in diaphragm BF during submaximal exercise (481 ml/min/100g; $P < 0.05$) via Grubbs' Test for Outliers and was removed from statistical analysis of diaphragm BF and VC. Statistical significance was set at $P < 0.05$.

3. Results

3.1 Blood glucose, body weight and heart morphometrics

Blood glucose levels were significantly higher while body weights were significantly lower in the GK rats compared to CON rats (Table 1). LVEDP, LV dP/dt, lung weight normalized to body weight were not different between GK and CON rats. Lastly, LV and RV weights normalized to body weight were significantly greater, whereas diaphragm weights and diaphragm weights normalized to body weight were significantly lower in GK compared to CON rats.

3.2 Hemodynamic responses

MAP was not different between GK rats and CON rats at rest (Table 2, $P = 0.95$) or during exercise ($P = 0.76$). HR was not different between GK and CON rats at rest ($P = 0.11$), or during exercise ($P = 0.50$).

3.3 Respiratory muscle BF

BFs at rest were not different between groups with respect to the diaphragm (Fig. 1A, $P = 0.50$), intercostals (Fig. 1B, $P > 0.99$), or transversus abdominis (Fig. 1C, $P = 0.89$). Diaphragm, intercostal and transverse abdominis BFs were significantly greater for both groups during submaximal exercise compared to rest ($P < 0.01$ for all). During submaximal exercise, diaphragm BF was higher in GK compared to CON rats (Fig. 1A, $P < 0.01$). Importantly, the higher diaphragm BF in GK compared to CON rats during exercise was present even when BF was expressed relative to 100g of body weight (CON: 0.40 ± 0.03 , GK: 0.47 ± 0.03 ml/min/100g body weight; $P = 0.02$). That finding suggests that the smaller diaphragm weight/body weight ratio in the GK rats was not the sole driver of the higher diaphragm BF when expressed as ml/min/100g of diaphragm weight as shown in Fig 1A. Further, GK, compared to CON rats had a greater increase in diaphragm BF during submaximal exercise from rest when compared to their CON counterparts (Fig. 2A; $P < 0.04$). During submaximal exercise BFs were not different between groups to the intercostals ($P = 0.24$) or transverse abdominis ($P = 0.83$). Moreover, the increase in BF was not different between groups to the intercostals (Fig. 2B, $P = 0.14$) or transverse abdominis (Fig. 2C, $P = 0.92$).

3.4 Respiratory muscle VCs

VCs at rest were not different between groups with respect to the diaphragm (Fig. 3A, $P = 0.47$), intercostals (Fig. 3B, $P > 0.99$), or transversus abdominis (Fig. 3C, $P = 0.85$). Diaphragm, intercostal and transverse abdominis VCs were significantly greater for both groups during submaximal exercise compared to rest ($P < 0.01$ for all). During submaximal exercise, diaphragm VC was higher in GK compared to CON rats (Fig. 3A, $P < 0.01$). Further, GK, compared to CON rats had a greater absolute increase in diaphragm VC during submaximal exercise from rest (Fig. 4A; $P < 0.01$). During submaximal exercise VCs were not different between groups to the intercostals ($P = 0.15$) or transverse abdominis ($P = 0.96$). Moreover, the increase in VC was not different between groups to the intercostals (Fig. 4B, $P = 0.08$) or transverse abdominis (Fig. 4C, $P = 0.84$).

3.4 Relationships

In GK rats, there was no linear relationship between blood glucose values and the change in BF during submaximal exercise from rest to the diaphragm ($r = 0.01$; $P = 0.98$) intercostals ($r = 0.17$; $P = 0.66$), or transverse abdominis ($r = 0.43$; $P = 0.06$). Further, there was no relationship in GK rats between the change in diaphragm BF from rest to submaximal exercise (*i.e.* Diaphragm BF) and lung weight normalized to body weight ($r = 0.35$; $P = 0.36$). Conversely, when data from GK and CON rats were combined, there were significant, negative correlations (Fig 5) between diaphragm weight and Diaphragm BF ($r = -0.61$; P

< 0.01), and between normalized diaphragm weight to body weight and the Diaphragm BF from rest to submaximal exercise ($r = -0.64$; $P < 0.01$).

4. Discussion

This investigation is the first to determine the effect of T2DM on respiratory muscle BF at rest and during submaximal exercise and revealed the following novel findings: 1) diaphragm muscle BF and VC are greater in the GK rat model of T2DM compared to healthy CON rats during submaximal exercise and 2) diaphragm muscle BF during submaximal exercise is negatively correlated with absolute diaphragm weights and diaphragm weights normalized to body weight. Our data have important implications for cardiovascular control during exercise in T2DM patients.

T2DM is associated with pulmonary alterations at rest and during exercise, but the subsequent impact on respiratory muscle BFs is unclear. Consistent with our hypothesis, we found that GK rats had a significantly higher diaphragm BF and VC during submaximal exercise compared to CON rats. In contrast to the diaphragm BF and VC, intercostal and transversus abdominis BFs and VCs were not different during submaximal exercise in GK rats compared to their healthy CON counterparts. The submaximal exercise diaphragm BF in CON rats reported herein (*i.e.* ~138 ml/min/100g) is in excellent agreement with submaximal exercise BFs reported in healthy Wistar, Sprague Dawley, and Fischer 344 X Brown Norway rats (110–124 mL/min/100g; Sexton and Poole, 1995; Smith et al., 2017a, b; Smith et al., 2019). This agreement provides confidence that the between-group difference in diaphragm BF during submaximal exercise is the consequence of a higher BF in GK rats.

The GK rat model of T2DM is known to develop cardiomyopathy, which may progress into overt heart failure (Korkmaz-Icoz et al., 2016). Importantly, previous studies from our laboratory have shown elevated diaphragm BFs during submaximal exercise in rats with congestive heart failure (CHF) which relates to the development of pulmonary congestion as indicated by increases in lung weights normalized to body weights (Musch, 1993; Smith et al., 2018; Smith et al., 2017a). Therefore, it was imperative to determine whether the GK rats in the present investigation had evidence of myocardial dysfunction and pulmonary congestion. Consistent with others (D'Souza et al., 2011; Korkmaz-Icoz et al., 2016), we found evidence of cardiac hypertrophy in GK rats as indicated by greater LV and RV weights normalized to body weights compared to CON rats. However, in GK rats we found no evidence of either LV dysfunction (*i.e.* no elevated LVEDP or decreased LV dP/dt), or pulmonary congestion (*i.e.* no elevated lung weight normalized to body weight). Moreover, we did not find a relationship in GK rats between lung weight normalized to body weight and the increase in diaphragm BF from rest to submaximal exercise. Thus, what is thought to contribute to the greater increase in diaphragm BF from rest to exercise in CHF rats (*i.e.* higher lung weight normalized to body weight ratio), does not appear to contribute to the greater increase in diaphragm BF from rest to exercise in the GK rat model of T2DM.

In the present investigation, we chose a workload which corresponds to ~55% of a healthy rat's $\dot{V}O_{2\max}$ (Musch et al., 1988). However, T2DM patients are known to have reductions in $\dot{V}O_{2\max}$ (Regensteiner et al., 2003). Thus, although not explicitly measured, the GK rats

were likely exercising at a higher relative intensity which could, at least in part, contribute to the higher diaphragm BF in GK rats compared to CON rats during submaximal exercise. Nevertheless, patients with T2DM and healthy individuals perform activities of daily living that are equivalent in absolute terms. Hence, we contend that comparing diaphragm BF between GK and CON rats at the same absolute work rate provides important information regarding cardiovascular regulation during exercise in T2DM. For example, if the maximum BF capacity to the diaphragm in GK rats is not increased, then the GK rats would have less diaphragm BF reserve compared to CON which could theoretically likely contribute to premature fatigue during severe intensity exercise.

What are additional potential underlying mechanisms responsible for the higher diaphragm BF during submaximal exercise in GK rats? First, in GK rats we found lower normalized diaphragm weights compared to CON rats. This finding is consistent with the lower lean muscle mass and respiratory muscle strength commonly reported in T2DM patients compared to healthy adults (Correa et al., 2011; Fuso et al., 2012; Kiely et al., 2014; Kim et al., 2014; Park et al., 2006). Moreover, this observation suggests that even in the presence of any possible pulmonary/ventilatory alterations that would have contributed to a greater work of breathing and thus diaphragm BF during submaximal exercise in GK rats, the lower diaphragm weights relative to body weight in GK rats would have acted to potentiate this diaphragm BF requirement. In further support of this notion, we found a negative relationship between the changes in diaphragm BF from resting values to exercising values and diaphragm weight (expressed as a function of total diaphragm weight or total diaphragm weight normalized to total body weight; see Fig. 5A and 5B). Second, in our previous study, we found that BF was higher to primarily glycolytic muscles of the hindlimb in GK compared to CON rats (Copp et al., 2010). This suggests an increased recruitment of glycolytic muscle fibers, which may have produced a greater $\dot{V}CO_2$, via HCO_3^- buffering of lactic acid-generated H^+ , thereby contributing to a larger ventilatory response during exercise in the GK compared to CON rats. Third, the GK rats may have experienced a higher $\dot{V}_E/\dot{V}CO_2$ during submaximal compared to CON rats, as is commonly reported in patients with T2DM patients (Gurdal et al., 2015; Roberts et al., 2018; Tantucci et al., 1996; Vukomanovic et al., 2019), which may have elicited a greater diaphragm energetic requirement and a concomitant increase in diaphragm BF. Finally, the work of breathing during exercise for a given ventilation may have been greater for GK rats compared to CON rats which if present would necessitate an accentuated diaphragm BF. In support of this notion, T2DM patients exhibit lower resting pulmonary function (Correa et al., 2011; Fuso et al., 2012; Huang et al., 2014; Klein et al., 2011; Shah et al., 2013) as described in the introduction. However, it is unclear if this results in greater work of breathing during exercise and also whether or not this occurs in the GK model of T2DM.

There are at least four experimental considerations which should be noted. First, we did not measure respiratory mechanics in our rats. Although it is unknown if GK rats experience pulmonary dysfunction, multiple reports provide strong evidence for pulmonary dysfunction in T2DM patients as mentioned previously (Anandhalakshmi et al., 2013; Chance et al., 2008; Correa et al., 2011; Fontaine-Delaruelle et al., 2016; Foster et al., 2010; Fuso et al., 2012; Huang et al., 2014; Klein et al., 2011; Saler et al., 2009; Shah et al., 2013; Tantucci et al., 1996; Weynand et al., 1999; Zineldin et al., 2015). Second, ventilation and $\dot{V}CO_2$ were

not measured at rest or during exercise in our rats, thus we do not know if GK rats had a greater $\dot{V}_E/\dot{V}CO_2$ compared to CON rats during submaximal exercise. However, T2DM patients are commonly reported to have an exaggerated ventilatory response to maintain alveolar ventilation (Gurdal et al., 2015; Roberts et al., 2018; Tantucci et al., 1996; Vukomanovic et al., 2019). Third, although the intercostals and transverse abdominis muscles are involved in ventilation in the quadruped (Robertson et al., 1977), they may also be involved in locomotion (Marlin et al., 2002; Poole et al., 2000). Finally, we did not investigate regional differences in diaphragm BF or VC. Future studies could elucidate whether the elevated diaphragm BF and VC during submaximal exercise demonstrated herein preferentially impact discrete regions of the diaphragm.

5. Conclusions

This investigation demonstrates that diaphragm BF and VC are greater during submaximal exercise in the GK rat model of T2DM compared to healthy CON. In addition, our data suggest that the smaller diaphragm weights in GK rats elicited a greater diaphragm BF requirement for a given diaphragm muscle weight, independent of any altered pulmonary/ventilatory requirements. In the absence of an augmented BF capacity of the diaphragm in T2DM this would reduce the BF reserve and potentially place the diaphragm closer to failure during severe intensity exercise. Moreover, greater diaphragm BF enhances the opportunity to “steal” that BF from the locomotor muscles (Harms et al., 1997), thereby compromising their performance.

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Highlights

- We measured diaphragm blood flow (BF) in type 2 diabetic rats and in controls
- At rest, diaphragm BF was not different between groups
- During submaximal exercise, diaphragm BF was higher in type 2 diabetic rats

Figure 1: Diaphragm (A), intercostal (B), and transversus abdominis (C) BF at rest and during submaximal exercise in CON ($n=8$; white bar) and GK ($n=10$; gray bar) rats. Diaphragm, intercostal, and transversus abdominis BF increased ($p < 0.02$) from rest to submaximal exercise. GK rats had greater ($P < 0.01$) diaphragm BF compared to healthy rats during submaximal exercise. *, $P < 0.05$ from rest; †, $P < 0.05$ GK vs. CON.

Figure 2:

Individual in diaphragm (A), intercostal (B), and transverse abdominis (C) blood flow (BF) from rest to submaximal exercise in CON rats ($n=8$; white bar) and GK rats ($n=10$; gray bar). GK rats had a significantly greater increase in diaphragm VC, but not intercostal VC or transverse abdominis VC compared to CON rats. * $P < 0.05$ GK vs. CON

Figure 3:

Diaphragm (A), intercostal (B), and transversus abdominis (C) VCs at rest and during submaximal exercise in CON ($n=8$; white bar) and GK ($n=10$; gray bar) rats. Diaphragm, intercostal, and transversus abdominis VCs increased ($p < 0.05$) from rest to submaximal exercise. GK rats had greater ($P < 0.01$) diaphragm VC compared to healthy rats during submaximal exercise. *, $P < 0.05$ from rest; †, $P < 0.05$ GK vs. CON.

Figure 4:

Individual in diaphragm (A), intercostal (B), and transverse abdominis (C) vascular conductance (VC) from rest to submaximal exercise in CON rats ($n=8$; white bar) and GK rats ($n=10$; gray bar). GK rats had a significantly greater increase in diaphragm VC, but not intercostal VC or transverse abdominis VC compared to CON rats. * $P < 0.05$ GK vs. CON

Figure 5:

Relationships between (A) diaphragm weight and the change in diaphragm BF from rest to submaximal exercise and (B) diaphragm weight normalized to body weight (diaphragm weight/body weight) and the change in diaphragm BF from rest to submaximal exercise in CON ($n=8$; open squares) and GK ($n=9$; closed circles) rats.

Table 1.

Weights, heart morphometrics and blood glucose values in CON and GK rats

	CON (<i>n</i> = 8)	GK (<i>n</i> = 10)	P-Value
Body weight (g)	516 ± 16	417 ± 14	<0.01
Blood Glucose (mg/dL)	103 ± 4	246 ± 29	<0.01
LVEDP (mmHg)	4 ± 1	5 ± 1	0.21
LV dP/dt (mmHg/s)	9,800 ± 920	10,389 ± 588	0.59
LV wt/body wt (mg/g)	2.12 ± 0.06	2.39 ± 0.06	<0.01
RV wt/body wt (mg/g)	0.52 ± 0.02	0.61 ± 0.01	<0.01
Lung wt/body wt (mg/g)	2.51 ± 0.09	2.81 ± 0.12	0.08
Diaphragm wt (mg)	1,473 ± 28	1,043 ± 39	<0.01
Diaphragm wt/body wt (mg/g)	2.88 ± 0.13	2.51 ± 0.07	0.03

Values are means ± SE. LVEDP, left ventricular end-diastolic pressure; LV dP/dt, left ventricular developed pressure; LV, left ventricle; RV, right ventricle; wt, weight. Data were compared using student's *t*-test.

Table 2.

MAP and HR at rest and during submaximal exercise in CON and GK rats.

	MAP (mmHg)	HR (bpm)
CON		
Rest	131 ± 5	372 ± 12
Exercise	139 ± 3	500 ± 6 *
GK		
Rest	133 ± 7	439 ± 19
Exercise	134 ± 5	506 ± 15 *

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate. Data were compared two-way ANOVA.

* P < 0.05 vs rest