The role of β2-adrenergic receptors in mediating sex differences in exercise hyperemia

by

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This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis.

This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

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Chapter 1: Connor J. Doherty drafted the chapter; Connor J. Doherty and Paolo B. Dominelli edited, revised and approved the final version of the chapter.

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Abstract

Cardiovascular sex differences have been identified between young males and females at rest. Young females typically exhibit lower resting blood pressure and reduced sensitivity to α-adrenergic receptor–mediated vasoconstriction compared with males. An upregulation or increased activity of β-adrenergic receptors has been proposed as a potential mechanism underlying these resting hemodynamic differences. However, it remains unclear whether sex differences exist in the blood flow and vascular conductance responses to dynamic exercise, and whether exercise hyperemia is influenced by sex-related differences in vascular β_2-adrenergic receptor activity. Thus, the purpose of this thesis was to examine the role of vascular β_2-adrenergic receptors in mediating the hyperemic response in males and females during dynamic isolated single-leg, forearm, and diaphragm exercise, both with and without systemic β-adrenergic receptor blockade using HCl propranolol. First, femoral artery blood flow to the active leg was assessed using Doppler ultrasound during progressive single-leg knee-extension exercise to exhaustion. Blood flow and vascular conductance responses to the active limb were not different between sexes and were not altered by propranolol. Second, brachial artery blood flow was measured during submaximal and fatiguing rhythmic handgrip exercise. Again, blood flow and vascular conductance responses did not differ between sexes or with β-adrenergic blockade. Notably, females exhibited a greater blunting of both femoral and brachial artery dilation with propranolol, suggesting a distinct contribution of vascular β_2-adrenergic receptors to exercise-induced vascular control in females. Third, contrast-enhanced ultrasound was employed to quantify diaphragm blood flow during mild-to-moderate intensity respiratory muscle exercise on an inspiratory resistance threshold device. Diaphragm blood flow and vascular conductance response to diaphragmatic work were not different between males and females and were unaffected by systemic β-adrenergic blockade. Together, these findings indicate that β-adrenergic receptors do not play a dominant role in mediating the overall exercise hyperemia response in to the leg, forearm and diaphragm. Instead, the results support the broader concept that exercise hyperemia is maintained through multiple redundant and compensatory mechanisms that ensure adequate blood flow and oxygen delivery to active skeletal muscle.

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When I first started my PhD, I was reminded of the "U-shaped" PhD experience, where a student's enjoyment begins high, decreases sharply as they navigate the rigours of academia, and then rises again near the end. As I approach the conclusion of my degree, however, I can't help but feel that "joy" is the wrong metric to describe that final upward trend. Instead, I believe that gratitude is what truly fuels it. Above all else, I am deeply grateful for the many people who have supported me throughout my PhD journey.

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List of Equations

(1) $\dot{Q} = \frac{\dot{V}O_2}{(CaO_2 - CvO_2)}$ 2

(2) $y = A(1 - e^{-Bt})$ 15

(3) $QMM = (Sex \times 267.7) + (MT \times 249.3) + (L \times 41.1) - 1663.7$ 32

(4) $CaO_2 = 1.34 \times hemoglobin \times SaO_2$ 37

(5) $\dot{Q}_{LEG} = MBV \times \pi \times FA\,radius^2 \times 60$ 37

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List of Abbreviations

%MIP – Mouth pressure relative to maximal inspiratory pressure
%PDI_{MAX} – Transdiaphragmatic pressure relative to maximal transdiaphragmatic pressure
ANOVA – Analysis of variance
AR – Adrenergic receptor
ATP – Adenosine triphosphate
AU – Arbitrary units
BA – Brachial artery
BA_D – Brachial artery diameter
BMI – Body mass index
CaO_2 – Arterial content of oxygen
CEUS – Contrast enhanced ultrasound
CO_2 – Carbon dioxide
CON – Control condition
COX – Cyclooxygenase
CvO_2 – Venous content of oxygen
DC – Duty cycle
DVC – Diaphragm vascular conductance
DVC_{MFR} – Diaphragm vascular conductance derived from microvascular flux rate
$DVC_{\dot{Q}DIA}$ – Diaphragm vascular conductance derived from diaphragm blood flow
ECG – Electrocardiogram
EMG – Electromyographic measurements
eNOS – Endothelial nitric oxide synthase
Est. O_2 del. – Estimated oxygen delivery
FA – Femoral artery
Fb – Breathing frequency
FEV_1 – Forced expired volume in one second
FEV_1/FVC – Ratio of forced expired volume in one second to forced vital capacity
FMD – Flow mediated dilation
FVC – Forced vital capacity
Hb – Hemoglobin
HCl - Hydrochloride
HR – Heart rate
IC – Inspiratory capacity
ICC – Inter-class correlation
ICG – Indocyanine green dye
ITL – Inspiratory threshold loading exercise
IUD – Intrauterine device
IV – Intravenous infusion
K^+ – Potassium ion
KE – Knee extension
LVC – Leg vascular conductance
MAP – Mean arterial pressure
MBV – Diaphragm microvascular blood volume
MBVel – Mean blood flow velocity

MFR – Diaphragm microvascular blood flux rate
MIP – Maximal inspiratory pressure
MSNA – Muscle sympathetic nerve activity
MT – Muscle thickness
MVC – Maximal voluntary contraction
nLVC – Normalized leg vascular conductance
$n\dot{Q}_{LEG}$ – Normalized leg blood flow
NIRS – Near infrared spectroscopy
NIRS-ICG – Near infrared spectroscopy with indocyanine green
NO – Nitric oxide
O_2 – Oxygen
P_{DIA} – Transdiaphragmatic pressure
P_{ESO} – Esophageal pressure
P_{GAS} – Gastric pressure
PI – Pulse interval
P_M – Mouth pressure
PROP – Propranolol condition
PTP – Pressure time product
PTP_{DIA} – Pressure time product derived from transdiaphragmatic pressure
$\dot{Q}$ - Cardiac output
$\dot{Q}_{ARM}$ – Arm blood flow
$\dot{Q}_{DIA}$ – Diaphragm arm blood flow
$\dot{Q}_{LEG}$ – Leg blood flow
QMM – Quadriceps muscle mass
RER – Respiratory exchange ratio
ROI – Region of interest
SaO_2 – Arterial oxygen saturation
TPR – Total peripheral resistance
TTI_{DIA} – Tension-time index of the diaphragm
VC – Vital capacity
VC_{ARM} – Arm vascular conductance
$\dot{V}CO_2$ – Carbon dioxide production
$\dot{V}CO_{2peak}$ – Carbon dioxide production during peak aerobic exercise
$\dot{V}_E$ – Minute ventilation
VI – Video intensity
$\dot{V}O_2$ – Volume of oxygen consumed
$\dot{V}O_{2peak}$ – Oxygen uptake during peak aerobic exercise
V_T – Tidal volume
WOB – Work of breathing
ΔFA_D – Change in femoral artery diameter
ΔTidal thickness – Change in diaphragm thickness during tidal breathing
α-AR – Alpha adrenergic receptor
α1-AR – Alpha 1 adrenergic receptor
α2-AR – Alpha 2 adrenergic receptor
β-AR – Beta adrenergic receptor

β1-AR – Beta 1 adrenergic receptor
β2-AR – Beta 2 adrenergic receptor

Chapter 1: Literature review

1.1 Introduction:

Exercising muscle will receive increases in blood flow commensurate with the increase in oxygen demand (1, 2). Indeed, blood flow delivery (i.e., oxygen delivery) increases linearly with work performed by skeletal muscles (2, 3). Many fundamental studies investigating the cardiorespiratory response to exercise in humans were solely performed in males, discounting any impact biological sex could have on the cardiorespiratory adjustments to exercise. Over the last ~30 years, a focus on the influence of biological sex in the development of cardiovascular disease and normal cardiorespiratory function has uncovered several important sex differences. For example, young females tend to be protected from cardiovascular disease, while post-menopausal females appear to be at higher risk of developing cardiovascular disease (4). In addition, relative to age-matched young males, females tend to have lower resting blood pressures, lower sympathetic activity, and reduced sensitivity to alpha-adrenergic receptor (α-AR) mediated vasoconstriction (5–9).

Differences in beta-adrenergic receptors (β-AR) populations or sensitivities between males and females due to influence of circulating female sex hormones (i.e., estrogen) appear to play a role in mediating these differences (8). Another example of a sex difference observed during exercise is the greater hyperemia to exercising muscles observed in females relative to males during single-leg knee extension exercise (10, 11). However, whether the difference in β-AR activity between males and females is responsible for sex differences in the hyperemic response is currently unknown. Sex differences also exist in the pulmonary system, with females tending to have smaller airways and lungs (12–14). During high-intensity aerobic exercise necessitating high ventilations, smaller airways in females lead to greater airway resistance and greater work of breathing (WOB) for a given ventilation (13, 15, 16). The combined impact of sex differences in

airway morphology and β-AR sensitivities impact female respiratory muscle perfusion is equally unknown.

Thus, the first goal of this document is to outline relevant literature on the cardiovascular and respiratory response to exercise and highlight sex differences in the cardiorespiratory response to exercise. Three studies are then presented to comprehensively address the role of β-AR in mediating differences in the exercise hyperemia response in locomotor and respiratory muscles between males and females.

1.2 Regulation of the cardiorespiratory response to exercise

The transition from rest to exercise increases the energy demand from active skeletal muscle (17). Quickly after starting submaximal exercise skeletal muscle energy demands are entirely met by aerobic adenosine triphosphate (ATP) production via oxidative phosphorylation (18). Thus, the primary factor determining blood flow or systemic cardiac output ($\dot{Q}$) is oxygen demand. This relationship is clearly outlined by the Fick equation:

$$(1) \quad \dot{Q} = \frac{\dot{V}O_2}{(CaO_2 - CvO_2)}$$

Where $\dot{V}O_2$ is the volume of oxygen consumed, CaO_2 is arterial content of oxygen, and CvO_2 is venous content of oxygen (1, 19). Therefore, an increased oxygen demand by the body with exercise will proportionally increase $\dot{Q}$ to ensure that tissue oxygen demand is met.

Autonomic reflexes

The cardiovascular response to exercise is regulated through the integration of feedforward signals from supramedullary brain regions (i.e., central command) and feedback from peripheral afferents including muscle mechano- and metaboreceptors, arterial and cardiopulmonary baroreceptors, and arterial chemoreceptors (20–27). These inputs are received and integrated

within the cardiorespiratory control centre of the brainstem, which regulates efferent parasympathetic and sympathetic outflow during exercise (28). Central command functions as a feedforward mechanism involving parallel activation of motor and cardiorespiratory centres, increasing heart rate (HR), peripheral vascular tone and $\dot{Q}$ through parasympathetic withdrawal and increased sympathetic activity (20, 21, 29). In parallel, the exercise pressor reflex contributes to the cardiovascular response through activation of group III/IV skeletal muscle afferents that respond to mechanical deformation and metabolite accumulation within active skeletal muscle (25, 26, 30, 31). Activation of these afferents increases sympathetic nerve activity, blood pressure and HR during exercise (26, 32–34). Arterial baroreceptors continue to regulate blood pressure during exercise but are reset to defend arterial pressure around a higher operating point (35, 36). This resetting is influenced by input from central command and group III/IV afferents during exercise (37–39).

These same neural mechanisms also contribute to the regulation of the ventilatory response during exercise. Central command increases minute ventilation ($\dot{V}_E$) through coordinated increases in tidal volume (V_T) and breathing frequency (Fb) (40). Meanwhile, afferent feedback from locomotor muscles provides additional modulation of ventilation according to exercise intensity (40–43). Studies using hypnosis demonstrated increases in ventilation and reductions in end-tidal partial pressure of carbon dioxide (CO_2) when participants imagines exercising at a heavy workload, supporting a feedforward role of central command in ventilatory regulation (41). Similarly, $\dot{V}_E$ scales with the anticipated exercise intensity (42), whereas blunting of type III/IV afferent feedback from active skeletal muscle reduces the ventilatory response to exercise. (43).

Efferent sympathetic regulation of the heart and smooth muscle tone

Adrenergic receptors are sensitive to catecholamines released from the nervous system as neurotransmitters (i.e., norepinephrine) or released into circulation from the adrenal medulla (i.e., epinephrine). Vascular smooth muscles are high in receptor density with α-ARs, while β_2-adrenergic receptors are present, but at much lower concentrations (44). Consequently, increases in circulating catecholamines predominantly result in vasoconstriction (44–46). As exercise intensity increases, circulating epinephrine concentrations and norepinephrine release rise alongside increases in sympathetic outflow (24, 47, 48). Sympathetically-mediated vasoconstriction is essential for the maintenance of arterial pressure during exercise by restraining excessive vasodilation within active skeletal muscle (49). Indeed, leg exercise performed by sympathectomy patients resulted in a critical drop in blood pressure (49). This mechanism can be observed in healthy individuals as vascular conductance to a maximally exercising leg was reduced during superimposed arm or respiratory muscle exercise, redistributing a fraction of $\dot{Q}$ to maintain perfusion pressure (50–53).

Functional sympatholysis

Functional sympatholysis describes the blunting of sympathetic vasoconstriction in active skeletal muscle, permitting greater increases in blood flow during exercise (54). Studies in humans confirmed the presence of sympatholysis using tyramine to stimulate the release of norepinephrine at rest and exercise (55, 56). As exercise intensity increased, so did the blunting of sympathetic vasoconstriction, and this related linearly to the metabolite production during exercise (56). The exact mechanism of functional sympatholysis is not fully elucidated. However, inherent differences in adrenergic receptor sensitivity to functional sympatholysis may play a critical role in fine tuning blood flow distribution to skeletal muscle during exercise (1). In a series of studies by Faber and colleagues, α_1-ARs in larger arterioles, upstream from capillary beds were found to

be resistant to sympatholysis (57–59). Conversely, downstream α_2-ARs closer to capillary beds became desensitized with increases in pH (60, 61). These studies suggest functional sympatholysis helps to mediate increases in blood flow to the active muscle while preserving systemic perfusion pressure.

Local regulation of blood flow in skeletal muscle

From the first contraction of a skeletal muscle, blood flow to the muscle is increased (1). This hyperemic response appears to be biphasic, consisting of an immediate feedforward response followed by a slower metabolite-mediated vasodilation to finely tune the response during sustained exercise (62).

Feedforward mechanisms

Rapid vasodilation at the onset of exercise is mediated by several local factors including potassium (K+) release, nitric oxide (NO) production and mechanical compression of the vasculature by contracting skeletal muscle (i.e., skeletal muscle pump) (63–68). Potassium released into the interstitial space during muscular contractions contributes to hyperpolarization of vascular smooth muscle, while NO also appears to contribute to the rapid vasodilation observed at the onset of exercise (63–67). The skeletal muscle pump, may augment venous return and increase the pressure gradient across the exercising muscle, contributing to increases in blood flow (68). However, during high intensity exercise, high muscle forces may reduce blood flow through vascular compression (69).

Feedback mechanisms

A form of feedback from the exercising muscle tissue must exist to inform how blood flow is distributed in the exercise hyperemia. Several vasoactive substances released from exercising muscle and the circulation contribute to this response. Extracellular adenosine and ATP concentrations increase in proportion to skeletal muscle work and are potent vasodilators (70). However, variability in vasodilatory responses to exogenous adenosine infusion suggests adenosine may not be essential for mediating exercise hyperemia (71). In contrast, ATP infusions can produce increases in blood flow comparable to those observed during exercise and may also contribute to functional sympatholysis by attenuating sympathetic vasoconstriction (72–74). The endothelium is now understood to be a major regulatory site of vascular tone and contributes to exercise hyperemia through the production of NO and prostacyclins (75–77). Mechanical and chemical stimuli associated with exercise activate endothelial nitric oxide synthase (eNOS) and cyclooxygenase (COX) pathways promoting vasodilation (77). Stimulation of endothelial β_2-ARs may further augment NO production during exercise (78).

Redundancy within the exercise hyperemia response

The existence of multiple mechanisms controlling the hyperemic response emphasizes the priority placed upon closely matching oxygen delivery to exercising muscles. Numerous studies have demonstrated the degeneracy within the system when they observe no change in the steady state hyperemia response during inhibition of a single vasoactive substance (e.g., K^+ depletion, prostacyclin inhibition) (79–81). Only through the inhibition of activity from multiple vasoactive substances do we observe marked reductions in, but not complete inhibition, of the hyperemic response (67, 82, 83). The preservation of the exercise hyperemia response when only the COX or eNOS pathway is blocked may be explained by interactions between the two pathways within

endothelial and smooth muscle cells. Prostacyclins can directly inhibit eNOS activity; thus, selective inhibition of eNOS activity may not significantly alter exercise hyperemia since NO production is blunted by prostacyclins (82, 84, 85).

1.3 The exercise hyperemia response

The large increase in oxygen delivery to exercising muscle is accomplished by synchronized adjustments from the respiratory, cardiovascular, and local skeletal muscle systems. Blood pressure increases due to elevations in $\dot{Q}$ and sympathetically mediated vasoconstriction of quiescent tissues. Locally, exercising skeletal muscle tissue experiences increases in temperature and CO_2 production and elevations in hydrogen ions, promoting greater extraction of oxygen from hemoglobin by inducing a rightward shift of the oxygen dissociation curve right (86). The substantial vasodilation of the exercising muscle results in significant increases in blood flow and oxygen delivery. Whole-body exercise is a poor model to evaluate local exercise hyperemia due to the limitations of $\dot{Q}$ at high exercise intensities and the ensuing competition for blood flow (50, 51, 87–89). Thus, studies evaluating vascular regulation often utilize small muscle mass exercise to ensure blood flow is not limited by $\dot{Q}$. Nonetheless, a similar linear relationship between oxygen delivery and muscular work exists during small muscle exercise such as single-leg extension or handgrip exercise (2, 10, 90–92).

Knee extension exercise

Skeletal muscle receives a relatively small portion of total $\dot{Q}$ at rest, approximately 20% (1). Direct measurements of blood flow to the resting quadriceps using thermodilution and Doppler ultrasound imaging found flows that range from ~150 to 400 ml/min, while vascular conductance remains relatively low (93, 94). In the transition to exercise, active skeletal muscle experiences

substantial increases in blood flow and vascular conductance while mean arterial pressure, cardiac output and heart rate increase with workload, but remain below maximal values observe during whole-body exercise (2, 10, 95, 96). Consequently, knee extension exercise provides an effective model to examine local vascular regulation without the substantial cardiac output limitations observed during whole-body exercise.

However, in studies who have reported leg blood flow ($\dot{Q}_{LEG}$) and leg vascular conductance (LVC) responses to knee extension exercise, there is a considerable amount of variability, likely reflecting differences in methodologies, study populations and exercise protocols. In general, Doppler ultrasound studies report lower absolute measures of $\dot{Q}_{LEG}$ and LVC when compared to studies performed with thermodilation (2, 10, 91, 95, 97–100). For example, at a submaximal workload of ~20W, $\dot{Q}_{LEG}$ ranged from 1.1-3.1 $L \cdot min^{-1}$ (10, 91, 97–99), while thermodilution studies reported values ranging from 2.1-3.9 $L \cdot min^{-1}$ (2, 95, 100). Differences in study populations could explain this discrepancy, as early studies only included comparatively fit male participants (2, 93, 96). Indeed, Richardson and colleagues reported peak $\dot{Q}_{LEG}$ approaching 9 $L \cdot min^{-1}$ in highly trained cyclists capable of achieving work rates well above 100W during knee extension exercise (96). Variability between studies may also reflect differences in knee extension ergometers, exercise stage durations, incremental workload increases and body position during exercise. In the seated position, blood flow increases linearly with work rate during knee extension exercise (2, 91), whereas the supine position blunts the blood flow response, possibly due to lower hydrostatic driving pressures (101).

Rhythmic handgrip exercise

Rhythmic handgrip exercise is an equally effective model for investigating local vascular regulation since it recruits a smaller muscle mass than single-leg knee extension. Previous work reporting resting brachial artery blood flow range from ~30-150 $mL \cdot min^{-1}$ (90, 102–104). Like knee extension exercise, blood flow and vascular conductance to the active forearm increases with exercise intensity to match the metabolic demands of the exercising muscle (90, 92, 105). Accompanying increases in forearm blood flow ($\dot{Q}_{ARM}$) and vascular conductance (VC_{ARM}) are associated with elevations in HR and mean arterial (MAP); however, these responses remain substantially lower than those observed during large-muscle-mass or whole-body exercise due to the relatively small amount of active tissue recruited during arm exercise (106). That said, when handgrip exercise activates the exercise pressor reflex, significant increases in HR and MAP can be observed (107, 108). In addition, considerable variability in absolute strength can impact local hemodynamics due to a greater compression of microvasculature in stronger individuals (69).

Work of breathing

Respiratory muscles are constantly active to maintain alveolar ventilation and sufficient blood oxygenation. With increased oxygen demand, greater respiratory muscle excursions are performed to produce higher airflows and thus greater alveolar ventilations. Work of breathing is the muscular effort to overcome the viscoelastic tissue forces and resistive airflow forces during respiration. Airway diameter plays a critical role in determining airflow resistance (109). The relationship between ventilation and WOB is curvilinear, whereby WOB increases exponentially for a given increase in $\dot{V}_E$ (110). At low ventilations, WOB increases in proportion to $\dot{V}_E$; however, at higher ventilations, resistance within the central airways increases and WOB rises disproportionately (15, 110). Therefore, respiratory muscle blood flow demand should increase exponentially as ventilation increases.

Respiratory muscle blood flow

Akin to locomotive skeletal muscle blood flow, respiratory muscles require commensurate increases in oxygen delivery as respiratory muscle work increases (88, 111). The diaphragm is the primary inspiratory muscle and forms a muscular partition between the thorax and abdomen (112). During inspiration, contraction of the diaphragm expands the thoracic cavity and generates negative intrathoracic pressure, facilitating airflow into the lungs. Diaphragm contraction also contributes to venous return through the respiratory muscle pump (113). The diaphragm may also possess distinct vascular properties and neural reflexes, having implications for local and systemic blood flow regulation.

A fundamental study by Aaker & Laughlin, found reduced α-AR sensitivity of diaphragm arterioles in mice, suggesting a blunted vasoconstrictor response to increased sympathetic activity and highlighting the importance of maintaining sufficient alveolar ventilation (114). However, work in rats found diaphragm arteries to be more sensitive to α-AR receptor agonists relative to locomotive skeletal muscle arteries (115). These contrasting findings may reflect differences in animal models and vessel generations examined, as α-AR distribution varies throughout the vascular tree. Larger arterioles exhibit greater α_1-AR populations with preserved vasoconstriction during muscular contractions, whereas downstream smaller arterioles contain more α_2-AR and display a blunted vasoconstrictor response during contractions (58, 60). Methodological limitations in assessing diaphragm perfusion have not allowed for this question to be investigated in humans.

There is evidence of a distinct respiratory metaboreflex mechanism which provides important feedback during exercise (53, 88, 116, 117). Increasing the WOB during exercise increases muscle sympathetic nerve activity (MSNA) and can alter blood flow and vascular

conductance to exercising tissues (52, 108, 118). Indeed, when simultaneous measures of respiratory muscle and quadriceps blood flow were examined during manipulations during near-max whole-body exercise, respiratory muscle blood flow increased and quadriceps blood flow was reduced with increases in WOB (116).

Compared to other respiratory muscles, the diaphragm receives a greater portion of $\dot{Q}$. Work by Manohar in ponies demonstrated that the diaphragm received 11.5 mL/min/100g of muscle while other respiratory muscles received 6 mL/min/100g of muscle at rest (119). In humans, resting measures of microvascular respiratory muscle blood flow from the 7th intercoastal space were found to be approximately 10 mL/100mL of tissue/min (3). Studies using near-infrared spectroscopy (NIRS) and indocyanine green (ICG) demonstrated increases in respiratory muscle perfusion with increasing respiratory muscle work (3, 120). However, this finding was not universal as others reported reductions in blood flow with increasing respiratory muscle work (121). Interpretation of these findings should be made cautiously, as contamination from underlying visceral tissues (i.e., liver) cannot be excluded. More recent using contrast enhanced ultrasound (CEUS) confirmed a linear relationship between respiratory muscle work and relative diaphragm perfusion in humans (111). Respiratory muscle perfusion during maximal whole-body aerobic exercise was found to be comparable to locomotive skeletal muscle perfusion. In ponies, diaphragm blood flow reached 261 mL/min/100g of muscle, compared with 249.7 mL/min/100g in exercising skeletal muscle (122). The portion of $\dot{Q}$ sent to the respiratory muscles in humans has been estimated to be ~10% in the untrained healthy individual (123), equivalent to ~2.5 $L \cdot min^{-1}$ assuming a maximal $\dot{Q}$ of 25 $L \cdot min^{-1}$.

1.4 Methods of measuring skeletal muscle blood flow

A number of techniques have been developed to assess skeletal muscle blood flow in humans. Early methods relied on indirect assessments of tissue using venous occlusion plethysmography (124, 125). Today, more advanced methods are available to directly quantify skeletal muscle perfusion such as dye dilution, thermodilution and Doppler ultrasound imaging (2, 91, 126–128). This section will place an emphasis on thermodilution and Doppler ultrasound due to their widespread application in studies of exercise hyperemia.

The gold standard in assessing skeletal muscle blood flow to exercising muscle is via constant infusion thermodilution. Thermodilution is an indicator technique, in which ice-code saline is infused rather than an indicator. A catheter is placed within the vein to infuse saline while temperature probes positioned upstream and downstream measure the resultant change in temperature in the blood. Blood flow is then determined from the temperature difference between the two probes, with larger temperature reductions indicating lower blood flow and smaller reductions reflective of greater blood flow. A major advantage of thermodilution is that it permits the assessment of blood flow during fatiguing whole-body exercise (2, 106). However, the technique is highly invasive, requiring venous catheterization, and repeater measurements may contribute to hemodilution (127).

Ultrasound Doppler imaging is a common method to assess skeletal muscle blood flow from measurements of conduit artery inflow to the muscle of interest. Simultaneous B-mode imaging and Doppler flow velocity records allow for the determination of vessel cross-sectional area and blood velocity, from which absolute blood flow can be determined. Ultrasound Doppler imaging is non-invasive and permits continuous assessment of blood flow during exercise. Its high temporal resolution makes it particularly useful for investigating rapid changes in blood flow, such

as the onset of exercise (91). In addition, ultrasound Doppler has been shown to produce low day-to-day variability (129). However, accurate measurements depend on maintaining stable probe positioning and are therefore limited to resting conditions or exercise models that minimized vessel movement (e.g., handgrip or knee extension exercise). Despite demonstrating good agreement with thermodilution (91), doppler ultrasound typically reports lower absolute blood flow values (10, 97–99).

Diaphragm blood flow

Due to the anatomical structure and location of the diaphragm, assessing diaphragm blood flow ($\dot{Q}_{DIA}$) is challenging. Notable work in exercising horses and dogs was performed using the radiolabelled microsphere technique, providing valuable insight into respiratory muscle perfusion during exercise (119, 122, 130–136). The radiolabeled microsphere technique is the gold standard to assess $\dot{Q}_{DIA}$; however, it requires post-mortem dissection of the animal and cannot be performed in humans.

Our understanding of $\dot{Q}_{DIA}$ in humans is almost entirely informed by work performed in animal models. Glaring anatomical and functional differences between humans and animal models likely lead to differences in diaphragm function and perfusion. First, anatomical differences between upright humans and quadrupedal animals alter the position of the diaphragm relative the heart and may influence diaphragm perfusion through differences in hydrostatic pressures. Second, because animals rely on limb movement to assist in thoracic expansion during exercise, the diaphragm likely plays a greater role in generating negative intrathoracic pressures in humans and likely receives a larger proportion of blood flow for a given respiratory effort. Prior to the development of CEUS assessment of $\dot{Q}_{DIA}$ (111, 137), only one study has directly assessed $\dot{Q}_{DIA}$ in humans using timed volume collection from the inferior phrenic vein (138). Unfortunately, the

highly invasive nature of this technique limits its application in standard human cardiorespiratory physiology research.

Direct measures of intercostal muscle blood flow have recently been obtained using echo-Doppler ultrasound imaging of the 8th intercostal space during resting breathing and mild respiratory muscle loading (139). However, this technique does not assess $\dot{Q}_{DIA}$, cannot differentiate between internal and external intercoastal muscle perfusion, and has not been applied during higher-intensity exercise (139). The most common method used to assess respiratory muscle perfusion in humans is the NIRS-ICG technique, which provides reliable measures of relative changes in microvascular perfusion within the region of interest (3, 120, 140–142). However, NIRS-ICG does not provide an assessment of bulk respiratory muscle blood flow and cannot distinguish between perfusion of the diaphragm, intercostal muscles, or underlying abdominal tissues. Consequently, differences in respiratory muscle recruitment (143, 144) and changes in blood flow to surrounding organs (1, 145) may influence estimates of respiratory muscle perfusion and contribute to the conflicting findings previously reported during exercise (140, 142). Given that the diaphragm performs the majority of inspiratory work (119, 122, 146), the inability of current techniques to isolate changes in diaphragm blood flow remains a major limitation in the study of respiratory muscle perfusion.

Contrast enhanced ultrasound

Contrast enhanced ultrasound imaging is a new method combining long-lasting contrast agents and high-resolution ultrasound technology to assess tissue microvascular perfusion. This method was initially developed to evaluate myocardial perfusion (147–149) but has since been used to assess the perfusion of tumours (150) and locomotive skeletal muscle (151–154) and now the diaphragm (111, 137). Contrast enhanced ultrasound imaging involves the intravenous infusion

of a contrast agent that is detected by the ultrasound transducer probe. In a process termed destruction-replenishment, blood flows of a specific tissue may be determined by measuring the inflow of a contrast agent into a region of interest following the destruction of the microsphere contrast agent by a strong ultrasound signal. The period after the elimination of the microbubble contrast agent for each image is termed the pulsing interval (PI) (147, 148, 155). The visual intensity (VI) is measured for each image and will provide a measure of the contrast agent density within the microvasculature of the diaphragm. Figure 1 depicts a burst replenishment curve that would develop following the destruction of microspheres. Blood flow may then be determined by fitting PI vs. VI to the following function:

$$(2) \quad y = A(1 - e^{-Bt})$$

Where y is VI at time t and A is the plateau intensity, reflecting microvascular blood volume (MBV) of the region of interest, B is the rate constant of microbubble replenishment and is

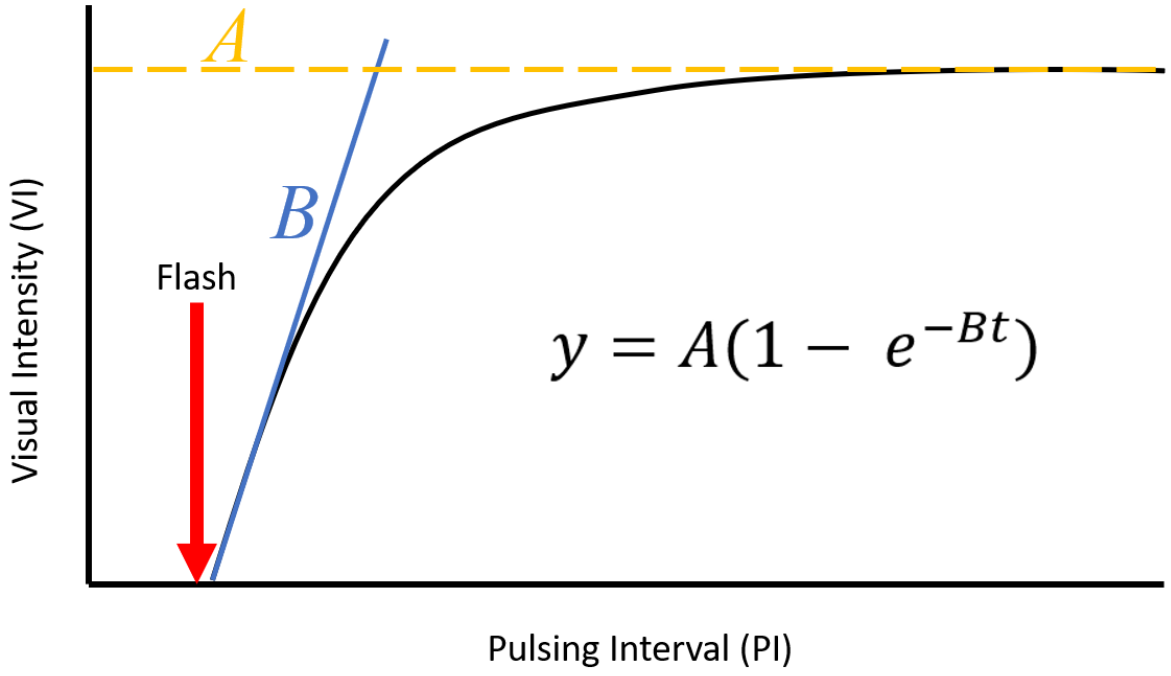


Figure 1. Burst replenishment curve following destruction of microspheres. A represents microvascular blood volume, B relates to microvascular blood velocity

proportional to the microvascular flux rate (MFR) of the tissue (111, 147, 148, 155). A relative measure of diaphragmatic microvascular blood flow ($\dot{Q}_{DIA}$) may then be quantified by the product of *A* and *B*.

The primary advantage of CEUS is that it allows for the direct assessment of $\dot{Q}_{DIA}$ in humans. During image analysis, the diaphragm is identified and isolated from surrounding tissues, including abdominal organs and superficial intercostal muscles. CEUS is safe and is widely used in clinical and research settings and the destruction-replenishment assessment of tissue perfusion permits multiple measurements during a single testing session. However, CEUS assessment of $\dot{Q}_{DIA}$ does have some limitations. First, measurements can only be obtained during end-expiratory apnea because the diaphragm must remain stationary during image acquisition. Consequently, CEUS does not provide continuous beat-to-beat measures of $\dot{Q}_{DIA}$., but rather assesses post-exercise hyperemia, similar to venous plethysmography measures of limb blood flow (156). Second, CEUS provides a measure of microvascular perfusion rather than bulk diaphragm blood flow. Therefore, differences in respiratory muscle recruitment (144, 157) could influence interpretation of overall respiratory muscle perfusion. Finally, although minimally invasive, CEUS requires venous catheterization for continuous infusion of the contrast agent.

1.5 Sex differences

Influence of sex hormones

Many physiological sex differences emerge following puberty, strongly implicating circulating sex hormones in the structural and functional differences observed between males and females (158, 159). In males, testosterone contributes to several cardiorespiratory adaptations, including greater hematocrit levels, and larger airway dimensions relative to females (159, 160). In young healthy adult females, circulating concentrations of estrogen and progesterone fluctuate across the

menstrual cycle (161). Although progesterone may influence vascular and ventilatory regulation, its role in mediating sex differences in cardiorespiratory function remains poorly understood (162–165)

In contrast, there is powerful evidence linking estrogen to sex differences in cardiovascular control. Estrogen appears to exert its actions through both central and peripheral mechanisms. In humans, resting MSNA is inversely related to circulating estrogen concentrations (5), while animal studies suggest estrogen may attenuate sympathetic outflow through nitric oxide-mediated inhibition of central sympatho-excitatory pathways (166, 167). At the level of the vasculature, estrogen promotes vasodilation through activation of endothelial estrogen receptors and the subsequent production of nitric oxide and prostacyclin (168). In addition to these acute effects, estrogen may exert longer-term influences on vascular function through modulation of endothelial β-AR populations (169) or enhancement of β-AR sensitivity via improvements in nitric oxide bioavailability (170).

Cardiovascular sex differences

On average, males have larger body size, blood volumes, and cardiac dimensions than females, resulting in greater resting and maximal cardiac outputs despite similar resting hear rates (1, 171, 172). As previously mentioned, males also have greater hematocrit and hemoglobin levels compared to females, with mean hemoglobin concentrations of ~15g/100ml in males and ~13g/100ml in females (173). Thus, greater hemoglobin concentrations in males contribute to higher arterial oxygen content and oxygen delivery capacity relative to females for a give volume of blood. Indeed, recent work found that males had ~10% increase in oxygen delivery, primarily explained by differences in arterial oxygen content (174).

Vasculature and hemodynamics

Sex differences in vascular function and hemodynamics have been linked to clinical outcomes and disease. For instance, females tend to have significantly lower blood pressures compared to males and have a greater chance of suffering from orthostatic intolerance (6, 7). However, the reduced resting blood pressures in young females may be cardioprotective as females have a significantly lower chance of developing cardiovascular disease (4). Arterial stiffness, a strong predictor of cardiovascular disease, is also greater in males relative to females (175). Males possess larger conduit arteries than females, including the aorta and common femoral artery (10, 176, 177). Young females also exhibit preserved endothelial, as evidenced by preserved flow-mediated dilation responses (178, 179). In contrast, postmenopausal females exhibit greater arterial stiffness and cardiovascular disease risk than age-matched males, suggesting the presence of sex hormones are critical to preserving vascular health to young healthy females (175).

The presence of estrogen in young females and its impact on overall sympathetic vasoconstrictor activity provides a possible explanation for the sex differences in blood pressure and hemodynamic regulation. In males an increase in sympathetic activity to peripheral blood vessels (i.e., MSNA) was found to be directly related to resting total peripheral resistance (TPR), and inversely related to $\dot{Q}$ (180). In contrast, no relationship was observed between MSNA and TPR, $\dot{Q}$ or MAP in young females, suggesting a difference in vasoconstrictor activity between the sexes (181). Consistent with these observations, young females exhibit attenuated α-AR-mediated vasoconstriction, whereas postmenopausal females display responses similar to young males (8, 9, 182). Females also exhibit greater β-AR mediated vasodilation (9, 182). The increased β-AR vasodilation in females may limit α-AR vasoconstriction as pre-treating forearm arteries with a β-blocker substantially increases vasoconstriction in young females, but not males (8, 9). Similarly,

Additionally, β-AR blockade restores the positive relationship between MSNA and TPR in young but not postmenopausal females (8). Collectively, these findings suggest sex differences in blood pressure regulation are mediated, in part, by differences in adrenergic receptor activity.

There is mounting direct and indirect evidence of sex differences in the exercise hyperemia response to exercising tissue. Indeed, female blood flow and vascular conductance response to fatiguing small muscle mass exercise is comparatively greater relative to males (10, 183–186). Greater blood flow and vascular conductance responses during intermittent handgrip exercise have consistently been observed in females relative to males (183, 185, 186). Similarly, females exhibit greater blood flow and vascular conductance during graded knee-extension exercise, even after normalizing for quadriceps muscle mass (10). In a follow up study, older females were found to have an attenuated blood flow and vascular conductance response relative to young females during knee extension exercise (11). Given the high sympathetic activity during maximal exercise, increased β-AR vasodilation interrupting α-AR vasoconstriction in young females is an appealing hypothesis that could explain the greater exercise hyperemia in females.

Studies examining sex differences in muscle fatigue and time to failure during fatiguing exercise provide some indirect evidence of differences in the exercise hyperemia response between males and females. Reduced oxygen delivery via blood flow restriction or hypoxia accelerates fatigue development (187, 188). Young females are understood to be more fatigue-resistant relative to their male counterparts (189). Even when matched for strength, eliminating the impact of muscle tension compressing the vasculature, females took longer to fatigue during intermittent handgrip exercise (190). Females also demonstrate greater fatigue resistance during knee extension exercise (188). Reduced fatiguability of the female diaphragm has also been observed, but this

might be explained by differences in the recruitment of respiratory muscles between males and females (191, 192).

Respiratory sex differences

Several sex differences in the anatomical structure of the respiratory system impacting physiological outcomes have been identified, particularly during exercise (12, 13). Adult males consistently exhibit lung volumes 10-15% greater than females, even after matching for age and height (193–195). Sex differences in thoracic morphology likely contribute to the larger lung volumes observed in males. Males typically have larger ribcages which are more pyramidal in shape with a wider base (193, 196, 197). These morphological sex differences in the rib cage can have implications for the diaphragm as females will tend to have a relatively smaller diaphragm (193).

The most consequential anatomical sex difference in the respiratory system is the smaller luminal area of large conducting airways in females (14). Evidence from studies employing dysanapsis measurements, radiographic imaging, acoustic reflectance, and computed tomography Large conducting airways are the site for the majority of airway resistance during respiration. Early evidence of sex differences in airway size was found when the dysanapsis ratio was found to be smaller in females compared to males (198–205). Importantly, these sex differences in airway size persist after correcting for sitting height (201, 203) and lung volume (204, 206). Sex differences in respiratory anatomy have important physiological consequences. Males generally achieve greater maximal ventilations and expiratory flow rates during exercise (12, 207). Because airway resistance is highly sensitive to airway radius, smaller conducting airways likely contribute to the lower maximal expiratory flows observed in females. Males also generate greater maximal respiratory pressures, likely reflecting greater respiratory muscle mass (208). In addition, females

exhibit lower pulmonary diffusion capacity than males, likely due to smaller lung volumes and reduced alveolar surface area (209, 210).

Sex differences in respiratory muscle energetics during exercise are primarily attributed to a greater WOB in females for a given $\dot{V}_E$ (13, 15, 16). Because the elastic component of WOB is similar between sexes, the greater WOB in females may be exaplned to an increase in resistive airflow arising from the relatively smaller conducting airways (15, 16). This sex difference becomes apparent at ventilations above ~ 60 $L \cdot min^{-1}$ (15, 199, 211). Interestingly, when the airway size was artificially increased with heliox, females displayed similar WOB as males breathing room air at ventilations of ~65 $L \cdot min^{-1}$, confirming differences in airway resistance drive the sex differences in WOB (212). Because oxygen delivery is closely matched to respiratory muscle work, the greater WOB in females is expected to increase respiratory muscle oxygen consumption ($\dot{V}O_2$) (88, 111). Consistent with this hypothesis, females demonstrate greater respiratory muscle $\dot{V}O_2$ and a higher relative oxygen cost of exercise hyperpnea than males, particularly at ventilations exceed 55-60 $L \cdot min^{-1}$ (123, 211, 213). Consequently, female respiratory muscles demand a greater proportion of $\dot{Q}$ during exercise than their male counterparts.

1.6 Effect of propranolol at rest and during exercise

Propranolol is a non-selective β-AR antagonist that inhibits the actions of epinephrine and norepinephrine of both β_1- and β_2- ARs, attenuating sympathetic nervous system activity through these pathways. Clinically, propranolol is widely used to treat a spectra of cardiovascular conditions, including hypertension, arrhythmias, angine, and myocardial infarction, as well as non-cardiovascular disorders such as migraines, essential tremor, and anxiety (214). In research, propranolol has become an important tool for investigating the role of β-AR in physiological processes included blood pressure regulation, metabolism and substrate utilization (8, 215–218).

To address the research questions of this thesis, complete β-AR blockade is required. Complete blockade is generally considered to occur when β-AR are saturated and blood propranolol concentrations exceed ~100 $ng \cdot mL^{-1}$ (219). However, complete blockade has also been shown to occur at blood concentration of 60-70 $ng \cdot mL^{-1}$ following oral administration, most likely due to the presence of an equipotent propranolol metabolite 4-hydroxypropranolol (220, 221).

Although propranolol is most commonly administered orally, there are some considerations that should be made when incorporated in an experimental design. Propranolol is rapidly absorbed and is widely distributed throughout tissues and peak concentrations occuring ~60-90 minutes following the dose and a half life of ~4 hours (222, 223). Oral propranolol undergoes extensive first-pass metabolism by the liver substantially reduces systemic availability, requiring repeated dosing to achieve target blood concentrations (219, 224). Consequently, there can be a large inter-individual variability in the bioavailability of orally administered propranolol, with similar doses producing markedly different blood propranolol concentrations (225). Finally, due to the chronic nature of taking oral propranolol it has often been associated with side effects including lassitude and hallucinations (226).

Intravenous administration of propranolol can overcome many of these challenges with oral propranolol as it allows for a controlled increase in blood propranolol concentrations, while avoiding differences in first-pass hepatic metabolism. The dosage scheme used by Bell *et al*. of an initial bolus 0.25 mg/kg over 5 minutes, followed by a 0.004 mg/kg/min continuous infusion will completely inhibit β-ARs (216). The initial bolus dose is critical to ensure β-ARs become fully saturated prior to maintaining the blockade with the continuous infusion dose. Importantly, this protocol has been utilized in numerous studies, and no reported adverse effects have been

associated with the protocol (8, 216, 227, 228). However, intravenously (IV) infused propranolol at doses required in the present thesis is significantly more expensive and requires the placement of a venous catheter, making it more invasive.

The cardiovascular impact of propranolol in humans is well-documented in the literature. At rest, decreases in HR by ~ 5-10 bpm are commonly observed following administration of propranolol (8, 218, 228–230). Resting blood pressure is generally maintained with IV propranolol by can be reduced by ~5mmHg with oral propranolol (8, 218, 229–231). The cardiovascular effects of propranolol become much more pronounced during exercise with heightened sympathetic nervous system activity. Indeed, blunted HR responses to exercise were found during isolated small muscle handgrip and single-leg cycling exercise (231–235). However, attenuated blood pressure responses were primarily observed during singe-leg cycling exercise, suggesting a greater muscle mass may be required to influence exercise blood pressure responses (232). The greatest reductions in HR and blood pressure responses to exercise were observed during high-intensity whole-body exercise (217, 218, 236–238). Significant reductions in peak HR (~30-50 bpm), MAP (~10-20 mmHg) and $\dot{Q}$ during with propranolol during exercise were associated with impaired exercise performance (217, 230, 237–239).

Propranolol will also impact smooth muscle tone of the airways. Because airway caliber at rest is primarily regulated by the parasympathetic nervous system (240), propranolol generally does not alter airway resistance in healthy individuals (215, 241, 242). However this finding is not universal as other work have reported increases in airways resistance following propranolol administration (243). Contrasting results may be attributed to the fact that β-AR are primarily located in smaller generation airways, but the majority of airway resistance occurs in larger

airways (244). During exercise, β_2-AR-mediated relaxation of airway smooth muscles improves airway conductance, an effect attenuated by propranolol (241, 245).

Chapter 2: Research question and hypotheses

The primary objective of this thesis is to investigate the role of β_2-ARs in mediating the difference in exercise hyperemia between females and males. The studies utilized measurements of blood flow with ultrasound imaging to quantify the exercise hyperemia response. Isolated skeletal muscle mass exercise (i.e., single-leg knee extension, rhythmic handgrip exercise and resistive inspiratory exercise) was performed to ensure that the exercise hyperemia response to the active muscle was not limited by cardiac output during exercise, particularly since non-selective β-AR inhibition reduces max cardiac output during exercise (217, 218, 246). Identical experimental protocols performed on two separate days will only differ by the administration of propranolol at a dose sufficient to cause complete β_2-AR blockade (219). Examining the changes in the blood flow and vascular conductance with the administration of propranolol between males and females will provide critical insight into the role of β_2-ARs in mediating the exercise hyperemia response. Detailed research questions and hypotheses are detailed in the introduction of Chapters 3, 4 and 5.

2.1 Overall research question

Are β_2-ARs in the vasculature responsible for the greater exercise hyperemia response to active skeletal muscle in females?

2.2 Overall hypotheses

1) A greater presence of β_2-AR is responsible for the greater blood flows and vascular conductance to active skeletal muscle in females.
2) Beta-adrenergic blockade with propranolol will attenuate blood flows and vascular conductance to exercising muscle to a greater extent in females compared to males.

Chapter 3: Role of beta-adrenergic receptors in mediating sex differences in the exercise hyperemia response

Doherty CJ, Thompson BP, Rynne PJ, Chang J-C, Pulford-Thorpe AE, Angus SA, Fleming ECM, Gartner SL, Au JS, Foster GE, Dominelli PB. The role of β-adrenergic receptors in mediating exercise hyperemia during incremental single-leg knee extension in young males and females. *Journal of Applied Physiology* 138: 1664–1679, 2025. doi: 10.1152/japplphysiol.00896.2024.

3.1 Introduction

Blood flow and oxygen (O_2) delivery are tightly matched to the work performed by the exercising skeletal muscle (1, 2). Exercise hyperemia is a complex integrated process regulated by numerous local and central mechanisms. The vital nature of preserving O_2 delivery to active skeletal muscle tissue is highlighted by the numerous mechanisms that exist in regulating exercise hyperemia (1, 67, 77, 247–249). At the level of active skeletal muscle, production of exercise metabolites rapidly vasodilates local resistance arterioles and feed arteries, increasing blood flow through upstream conduit arteries (1). Central regulation of the exercise hyperemia response occurs through changes in cardiac output and alterations in systemic vascular tone via modulation of parasympathetic and sympathetic nerve output (1). With increases in efferent sympathetic nerve activity to the peripheral vasculature, norepinephrine is released at the neuromuscular junction (78). Norepinephrine primarily binds to alpha-adrenergic receptors mediating an increase in the vasoconstriction of the vessel. However, norepinephrine also binds to beta 2 adrenergic receptors (β_2-ARs), which when stimulated can cause the relaxing of smooth muscle surrounding the vasculature (78).

Numerous sex differences have been identified in the regulation of the cardiovascular system at rest and during exercise (6, 8–10, 13, 181, 250, 251). At rest, females generally have lower blood pressure (250, 251), exhibit no relationship between sympathetic nerve activity and total peripheral resistance or cardiac output (181), and have a reduced sensitivity to alpha-

adrenergic receptor-mediated vasoconstriction (8, 9). In addition, young females may have a greater hyperemia response to single-leg knee extension exercise compared to their male counterparts (10). After normalizing for muscle mass, females had an even greater vascular conductance and hyperemic response to the leg relative to their male counterparts during dynamic knee extension exercise (10). A follow-up study comparing young and older females found vascular conductance and blood flow responses were blunted in older females relative to young females (11). However, sex differences in exercise hyperemia are not universal, as others demonstrated that forearm exercise did not result in any observable sex differences in blood flow or vascular conductance responses (252). Whether these equivocal findings are due to different exercise modalities and the magnitude of active skeletal muscle is not yet known.

A proposed mechanism responsible for sex-differences in blood pressure regulation at rest is the upregulation of β_2-ARs in females due to the greater presence of estrogen (253). Hart *et al.* provided experimental evidence for this by demonstrating that the administration of propranolol, a non-selective β-adrenergic antagonist, in females increased vasoconstrictor sensitivity to levels observed in males and post-menopausal females (8). Kneale *et al.* also found that β-adrenergic blockade with propranolol improved vasoconstrictor response to norepinephrine in females (9). Other work examining blood flow and vascular conductance responses to the exercising forearm during the infusion of propranolol found β-AR mediated vasodilation was reduced as exercise intensity increased, suggesting it may not be a critical mechanism mediating exercise hyperemia (233, 254). However, these studies were predominantly performed in males, and it remains unknown whether β-ARs play a role in mediating the increased exercise hyperemia response observed in females.

Accordingly, we sought to determine the impact of β-AR blockade on blood flow and vascular conductance to the exercising leg during knee extension exercise to failure in young males and females. We hypothesized that: (1) without β-AR blockade, females will have greater relative increases in blood flow and vascular conductance to the exercising leg compared to males during knee extension; and (2) following β-AR blockade, females will experience greater attenuations in leg blood flow and vascular conductance compared to males during single-leg knee extension exercise.

3.2 Methods

Sample size

Sample size was based on a previous study examining sex differences in vascular conductance during knee extension exercise (10). Cohen's f effect size from this study was determined to be 0.45. For a given alpha of 0.05 and a power of 0.80 this suggests a sample size of 7 per group for a total sample size of 14 was recommended to perform the study.

Participants

Sixteen healthy young adults (8 females; age, 26 ± 3 years [mean ± SD]) with a body mass index of 24.1 ± 2.2 kg/m^2 were recruited. Table 1 outlines baseline participant characteristics. Female participants were regularly menstruating (average length 26-32 days) (n=4) or using hormonal intrauterine device (IUD) (n=4). Both experimental testing visits in regularly menstruating female participants were performed during a similar menstrual phase 48-120 hours apart. (i.e., low-hormone early follicular phase or high-hormone mid-luteal phase). Of the regularly menstruating female participants, 2 were tested during the early follicular phase and 2 were tested during the mid-luteal phase. Females using hormonal IUD were tested at their convenience. Participants were excluded if they had cardiovascular, respiratory, metabolic, or any other diseases

that would impact their response to exercise. Participants were non-smokers and not taking any medication that would interact with propranolol. In addition, participants were restricted from performing exercise, consuming recreational drugs and consuming alcohol 24 hours prior to testing visits. The study was approved by the Clinical Research Ethics Board at the University of Waterloo (#44232) and adheres to the *Declaration of Helsinki*¸ except for registration in a database. All participants were informed of experimental procedures, potential risks and provided written, informed consent.

Table 1. Chapter 3 descriptive characteristics and peak exercise test.

Participant characteristics	Females (n=8)	Males (n=8)	*P*-value
Age (y)	25 ± 3	28 ± 5	**0.14**
Height (m)	1.66 ± 0.07	1.78 ± 0.06	**< 0.01**
Body mass (kg)	66.6 ± 9.8	78.6 ± 10.8	**0.02**
Body mass index ($kg \cdot m^{-2}$)	23.7 ± 1.6	24.7 ± 2.8	**0.39**
Quadriceps muscle mass (g)	1490 ± 161	1887 ± 259	**< 0.01**
Peak Exercise			
Power output (W)	241 ± 35	324 ± 65	**< 0.01**
Heart rate (bpm)	187 ± 10	187 ± 7	**0.99**
Ventilation ($L \cdot min^{-1}$)	121 ± 23	170 ± 33	**< 0.01**
Absolute $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	2.96 ± 0.44	4.0 ± 0.70	**< 0.01**
Relative $\dot{V}O_{2peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	45.3 ± 4.8	50.6 ± 7.4	**0.11**
$\dot{V}CO_{2peak}$ ($L \cdot min^{-1}$)	4.7 ± 0.8	3.5 ± 0.4	**< 0.01**
Respiration exchange ratio	1.19 ± 0.07	1.18 ± 0.04	**0.77**

Values are means ± SD. $\dot{V}O_{2peak}$, peak oxygen uptake; $\dot{V}CO_{2peak}$, peak expired carbon dioxide. P-value < 0.05 indicates significant effect of sex. Statistical analysis: unpaired Student's *t*-tests.

Experimental overview

Each participant completed four testing visits, with each visit separated by a minimum of 48 hours. During visit 1, participants performed a maximal exercise test on a cycle ergometer and had anthropometric measurements taken. In the second visit, participants were familiarized with the single-leg knee extension exercise protocol performed in subsequent testing visits. A single-

leg knee extension exercise model was selected to ensure exercise hyperemia responses would not be limited by the reductions in cardiac output during systemic β-AR blockade. During visits 3 and 4, participants completed the experimental testing protocol while continuous cardiorespiratory variables were assessed during single-leg knee extension to volitional fatigue with intravenously infused propranolol hydrochloride (HCl) (PROP) or without (control, CON) (Figure 2). The order between CON and PROP visits was randomized between participants, stratified by sex.

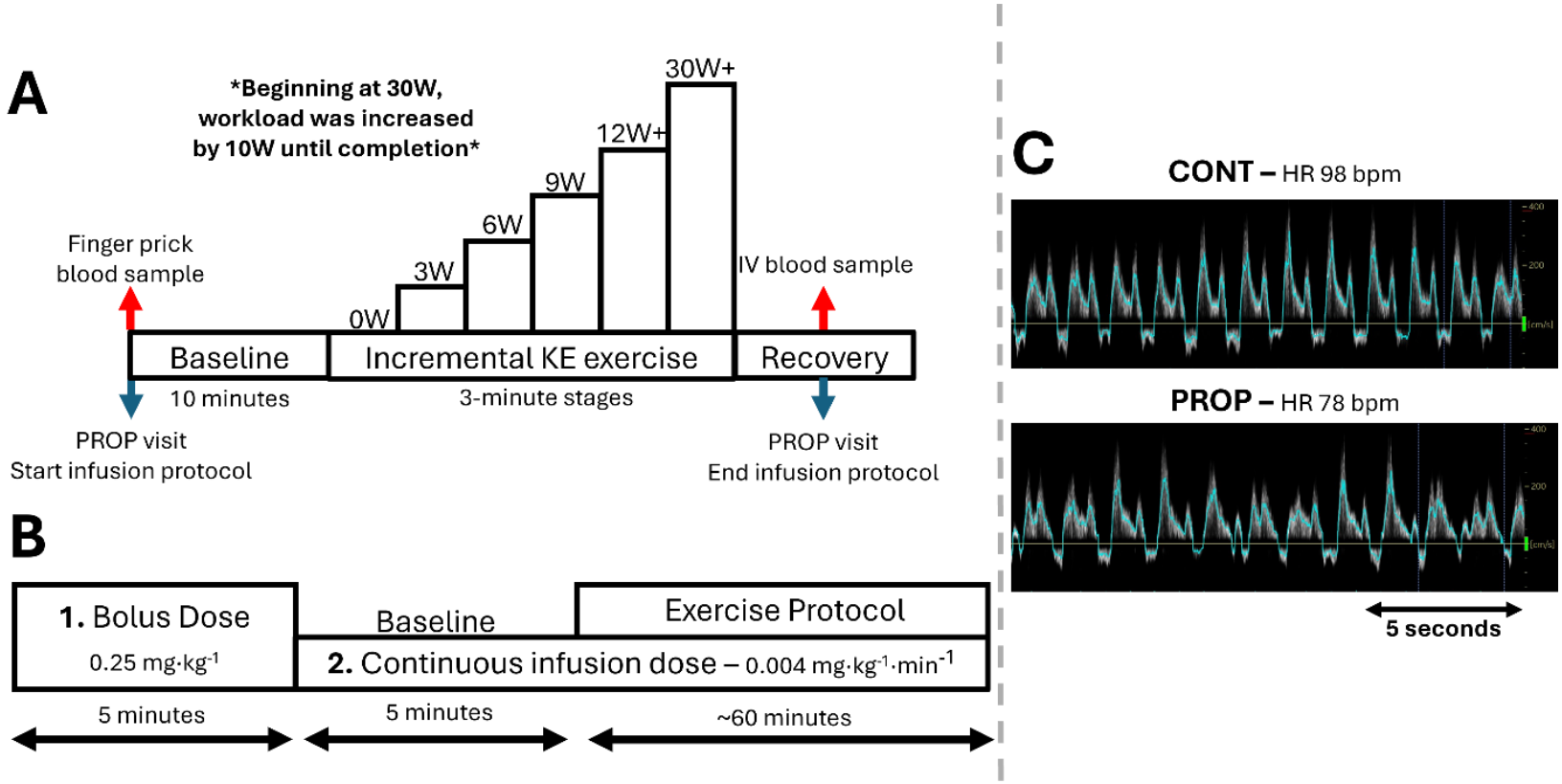


Figure 2. Experimental protocol, intravenous infusion protocol and representative femoral artery flow velocity tracings.

(**A**) single-leg knee extension protocol, (**B**) propranolol infusion protocol, and (**C**) representative femoral artery blood velocity profile at 30W of a single participant during the control and propranolol visit. Bolus dose (0.25 mg/kg) was provided over 5 minutes and was immediately followed by a continuous infusion dose (0.004 mg/kg/min) until completion of study. KE, knee extension; PROP, propranolol; IV, intravenous; CONT, control.

Anthropometrics

During visit 1, anthropometric measurements of height and body mass were taken, and body mass index was derived. Quadriceps muscle mass of the right leg (active limb) was estimated using an ultrasound-derived prediction of anterior thigh muscle volume (255). Briefly, femur length was measured from the greater trochanter to the lateral condyle of the right leg. Next, three ultrasound measures of quadriceps muscle thickness at the mid-thigh level were averaged together. Quadriceps muscle mass (QMM) was then calculated using the following equation:

$$(3) \qquad QMM = (Sex \times 267.7) + (MT \times 249.3) + (L \times 41.1) - 1663.7$$

where *Sex* is a factor (1 if male, 0 if female), *MT* is anterior thigh muscle thickness (cm), and *L* is thigh length (cm). This permitted individual leg blood flow ($\dot{Q}_{LEG}$) and leg vascular conductance (LVC) responses to be normalized to a participant's estimated quadriceps muscle mass.

$\dot{V}O_{2peak}$ test protocol. Participants performed a maximal cardiorespiratory exercise test on a cycle ergometer (LC7TT, Monark Exercise AB, Vansbro, Sweden). Detailed methods can be found elsewhere (256). Briefly, participants completed a ramped protocol starting at a resistance of 40 W for females and 60 W for males, with increases of 20 W/min until exhaustion. Measures of heart rate (HR) (Model T34; Polar Electro Oy, Kempele, Finland), mixed expired O_2 and carbon dioxide (CO_2) ((S-3-A/I and CD-3Am, Applied Electrochemistry, Bastrop, TX, USA) and ventilation ($\dot{V}_E$) (model 3813; Hans Rudolph, Shawnee, KS, USA) were collected continuously, allowing for the determination of peak O_2 consumption during exercise ($\dot{V}O_{2peak}$).

After completion of the maximal exercise test, participants were familiarized with the knee extension ergometer. Participants lay comfortably on the leg ergometer in a supine position with their knees bent at a 90° angle. The knee was positioned to be in line with the fulcrum of the knee

extension ergometer, and the leg strap was placed just above the ankle of the participant. During familiarization with the ergometer, ultrasound imaging of the participant's femoral artery (FA) in the active limb was performed. A previous pilot study conducted in 8 participants (4 females) examined the interclass correlation coefficient (ICC) of $\dot{Q}_{LEG}$ measures at rest and during 5 stages of exercise up to 12 W of the primary ultrasound technician (C.J.D.). At rest, ICC of $\dot{Q}_{LEG}$ measures were found to be 0.91, while exercise ICCs were on average 0.92 ± 0.02, with only 0W (0.89) having an ICC less than 0.9. Therefore, reliability of scanning was found to be excellent at rest and during exercise, according to previous guidelines (257).

Knee extension exercise protocol

During visits 2, 3 and 4, participants performed a knee extension exercise to volitional fatigue while continuous measures of cardiorespiratory variables, active leg muscle activity and ultrasound imaging of the FA were performed (Figure 2A). Experimental testing visits 3 and 4 were identical to the familiarization visit apart from a capillary blood sample collection taken to determine hemoglobin (Hb) concentration, and intravenous propranolol was provided to the participant throughout the PROP visit to systemically block β-ARs.

The knee extension ergometer was configured to allow the participant to work against a resistance during knee extension, while the spinning flywheel passively returned the leg to the starting position between kicks without the activation of other muscle groups. Participants were provided verbal instructions at the start and reminders throughout the exercise to limit activation of the hamstring muscle group throughout the exercise. After instrumentation, participants laid supine on the knee extension ergometer, and three minutes of quiet resting baseline were collected. Following baseline, the fly wheel of the ergometer was engaged by a researcher, and the participant began the exercise protocol. Single-leg knee extension exercise was completed at 40 kicks per

minute at a duty cycle of 1:1 for every kick and relaxation phase, a commonly used cadence during knee extension exercise (10, 11). Participants moved their leg from the relaxed position of 90° to an extended position of 160-170°. The initial exercise intensity was 0W and increased in a stepwise manner by 3W every three minutes until the participant was no longer able to maintain a kicking cadence. This workload was selected as it ensured that multiple stages could be completed by both male and female participants since previous studies performing incremental knee extension exercise a number of participants who could not achieve workloads greater than 20W (10). If a participant achieved 30W and was able to continue kicking, workload was then increased by 10W every three minutes until the participant was no longer able to maintain the kicking cadence (Figure 2).

Propranolol administration

Prior to baseline in the PROP visit, complete β-AR blockade was achieved using intravenous infusion of propranolol (Figure 2B). A 25 mL syringe containing 2 mg/mL propranolol was inserted into an infusion pump (PHD ULTRA Syringe pump, Harvard Apparatus, Holliston, MA, USA) and connected to microbore tubing feeding a 22-gauge catheter placed in the antecubital vein of the participants' left arm. An initial bolus dose of 0.25 mg/kg propranolol HCl was infused over 5 minutes. After completion of the bolus dose, propranolol HCl was continuously infused into the participant at 0.004 mg/kg/min to maintain β-AR blockade. The same IV propranolol infusion protocol has been used in numerous studies and previously demonstrated complete beta-adrenergic blockade in adult humans (216, 227). Approximately 30 minutes after the conclusion of the PROP visit, a blood sample was taken and a commercial ELISA kit (Propranolol Forensic ELISA Kit, NEOGEN, Lansing, MI, USA) was used to estimate blood

propranolol concentrations. Due to technical reasons, blood samples were not obtained in 2 male participants.

Cardiopulmonary measurements during knee extension

Expired flow was measured with a heated calibrated pneumotachometer to 37°C (model 3813; Hans Rudolph, Shawnee, KS, USA) from which tidal volume, breathing frequency and $\dot{V}_E$ were derived. Tidal volume and $\dot{V}_E$ are expressed as body temperature saturated with water vapor at ambient pressure. Participants wore a face mask and breathed through a three-way valve (Hans Rudolph) and large bore tubing with expired gases collected in a mixing chamber. Expired O_2 and carbon dioxide (CO_2) concentrations were measured with calibrated gas analyzers (S-3-A/I and CD-3Am, Applied Electrochemistry) from a sample line connected to the mixing chamber. The Haldane transformation was then used to derive inspired ventilation from $\dot{V}_E$ and known gas concentrations. Oxygen uptake and CO_2 production were calculated for each exercise intensity and expressed in standard temperature pressure and dry conditions.

Continuous measurement of HR was performed via electrocardiogram (ePM 12M, Mindray Bio-Medical Electronics, Shenzhen, China) while continuous beat-to-beat blood pressure was collected by finger photoplethysmography placed on the participants' left hand (Human NIBP Nano Interface; ADInstruments, Colorado Springs, CO, USA). Absolute discrete measures of blood pressure at rest were taken from an automated oscillometric sphygmomanometer placed around the upper right arm of the participant (ePM 12M). Electromyographic (EMG) measurements of the quadriceps and hamstring muscle groups were collected via surface electrodes (BMA-400 AC/DC Bioamplifier; CWE Incorporated, Ardmore, PA, USA). Skin on the distal quadriceps and hamstring muscle was shaved and cleansed with rubbing alcohol and a skin

preparation gel (Nuprep Skin Prep Gel, Weaver and Company, Aurora, CO, USA). Quadriceps muscle activity was measured by EMG surface electrodes on the vastus lateralis, while hamstring muscle activity was measured from EMG electrodes placed on the biceps femoris (258, 259). Reference electrodes were placed onto the patella with sufficient space to ensure they did not touch. The raw EMG signal was amplified 500x, bandpass-filtered with high-pass frequency of 25Hz and low-pass frequency of 100Hz. The root mean square of the filtered EMG signal was calculated to produce a mean voltage output signal.

Doppler ultrasound blood flow

Blood flow measurements of the right FA were made in the exercising leg by duplex-mode ultrasound (GE Vivid S70N; GE Healthcare, Chicago, IL) operating at 8MHz for B-Mode and 3.3 MHz for Doppler from a site just below the inguinal ligament, at least ~2 cm proximal from the bifurcation of the superficial and deep femoral arteries. Continuous measurements of both FA diameter and blood velocity profiles were captured, with the latter collected at an insonation angle of <60°. Images of the artery diameter were stored as video files on a computer and later analyzed offline using EchoPAC software (GE Healthcare, Chicago, IL). Artery diameters were analyzed by a single technician (C.J.D.) and were determined from the intima-lumen interface at both the distal and proximal vessel walls at end-diastole during the relaxation phase of the duty cycle. Images affected by motion artifact were excluded based on visual inspection by the technician. Time averaged mean blood velocities were determined using EchoPAC software.

Prior to the CON and PROP visits, a finger prick blood sample was taken to determine Hb concentration. Briefly, the finger was cleansed with an alcohol wipe and allowed to dry. A single-use lancet was used to make a small puncture in the finger and a 100 μL capillary blood sample was collected. Blood samples were analyzed with a blood analyzer (ABL80 FLEX CO-OX,

Radiometer, Copenhagen, Denmark). Estimated arterial O_2 content was calculated with the following equation:

$$(4) \qquad CaO_2 = 1.34 \times hemoglobin \times SaO_2$$

Where C_aO_2 is arterial O_2 content in the blood, and S_aO_2 is arterial O_2 saturation (assumed to be 97% due to lack of desaturation during maximal exercise). Due to the low solubility of O_2, the amount of dissolved O_2 in the blood is negligible and was ignored when calculating C_aO_2 and estimating O_2 delivery.

Data analysis

All respiratory and cardiovascular variables were calculated as 30s averages during the final minute of baseline and each exercise stage. All continuous signals were collected at 1000 Hz and recorded using PowerLab (AD Instruments, Sydney, NSW, Australia). Leg blood flow was calculated as:

$$(5) \qquad \dot{Q}_{LEG} = MBV \times \pi \times FA\,radius^2 \times 60$$

Leg vascular conductance was calculated as the quotient of $\dot{Q}_{LEG}$ and MAP. Normalized leg blood flow ($n\dot{Q}_{LEG}$) and normalized leg vascular conductance (nLVC) values were calculated by dividing $\dot{Q}_{LEG}$ and LVC by the participant's estimated quadriceps muscle mass. Oxygen delivery to the leg was estimated from the product of $\dot{Q}_{LEG}$ and CaO_2. Respiratory exchange ratio (RER) was calculated as the quotient of $\dot{V}CO_2$ and $\dot{V}O_2$. Femoral artery diameters were reported as the percent change in diameter (ΔFA_D) relative to the resting baseline diameter of the respective experimental

visit. Shear rate was calculated by: (8 × MBV)/ FA diameter. Muscle activity of the quadriceps and hamstrings was assessed as a percent increase from the 0W condition. All cardiorespiratory and local tissue perfusion variables were assessed relative to the maximal achieved workload during the control visit.

Statistical analysis

All statistical analyses were performed using GraphPad Prism Software (GraphPad Prism 10.4.1, La Jolla, CA, USA). Sex differences in participant characteristics, anthropometric measures, $\dot{V}O_{2peak}$ test results were assessed using unpaired Student's t-tests. Baseline and peak exercise intensity cardiorespiratory and local perfusion variables were assessed separately using a 2-way analysis of variance (ANOVA) (sex X propranolol) with *post hoc* Bonferroni-corrected Student's t-tests. Cardiovascular, respiratory and local tissue perfusion variables over absolute workloads from 0 – 15W and relative to peak exercise intensity 0 – 80% were assessed using 3-way ANOVA analysis (sex X propranolol X exercise intensity). A summary of main effects of sex, propranolol and exercise intensity and the (propranolol X sex) interaction may be found at the top of each figure. To eliminate exercise intensity as a factor and more closely examine the relationship between sex and β-AR blockade with propranolol, the individual slope relationship between each variable and workload was determined for each participant and analysed using a 2-way ANOVA (sex X propranolol) with *post hoc* Bonferroni-corrected Student's t-tests. Previous work found the increase in blood flow plateaus at higher exercise intensities during supine single-leg knee extension (101). Therefore, for $\dot{Q}_{LEG}$, $n\dot{Q}_{LEG}$, estimated leg O_2 delivery, LVC and nLVC variables, slopes were only derived from the linear portion of the variable-workload relationship and did not include exercise intensities once a plateau was achieved by the participant. Similarly,

individual slopes for each participant were derived for each cardiorespiratory and local tissue perfusion variable relative to maximal exercise intensity up to 80% and analyzed using a 2-way ANOVA (sex X propranolol) with *post hoc* Bonferroni-corrected Student's t-tests. Finally, males and females were pooled to statistically compare quadriceps and hamstring muscle activity due to signal loss in quadriceps EMG in 4 individuals (2 females) and hamstring EMG in 5 participants (3 females). Relative quadriceps (n=12) and hamstring activity (n=11) from 3-18W were assessed with a 2-way ANOVA (exercise intensity x propranolol) with *post hoc* Bonferroni-corrected Student's t-tests.

3.3 Results

Participant characteristics

Anthropometric measures of height, body mass and quadriceps muscle mass were greater in males (all $P < 0.05$). During the $\dot{V}O_{2peak}$ exercise protocol, males achieved a greater peak power output, $\dot{V}_E$, absolute $\dot{V}O_{2peak}$, and $\dot{V}CO_{2peak}$ values (all $P < 0.01$). Relative $\dot{V}O_{2peak}$, HR and RER values were not different (all $P > 0.10$). For CON, sample size for males was $n = 8$ until 24 W, $n = 6$ at 27 W, $n = 5$ at 30 W, $n = 3$ at 40 W, $n = 2$ at 50 W, and $n = 1$ at 60 W; CON sample size for females was $n = 8$ until 18 W, $n = 7$ at 21 W, $n = 4$ at 24 W, and $n = 1$ at 27, 30, 40, and 50 W. For PROP, sample size for males was $n = 8$ until 24 W, $n = 5$ at 27 W, $n = 4$ at 30 W, $n = 3$ at 40 W, $n = 2$ at 50 W, and $n = 1$ at 60 W; PROP sample size for females was $n = 8$ until 15 W, $n = 7$ at 18 W, $n = 6$ at 21 W, $n = 5$ at 24 W, and $n = 1$ at 27, 30, 40, and 50 W.

Propranolol blood concentrations

Males and females received total propranolol amounts of 40.2 ± 5.6 and 33.8 ± 6.0 mg, respectively. Estimated propranolol blood concentrations were not different by sex ~30 min after

the cessation of leg exercise (128.6 ± 16.2 vs. 114.9 ± 24.4 ng/mL; $P = 0.23$). Complete systemic β-adrenergic blockade occurs at propranolol blood concentrations greater than 100 ng/mL (219, 246). Only 2 male participants in the presents study had propranolol concentrations less than 100 ng/mL (87 and 94 ng/mL). The two males that were minimally under the threshold had blood taken ~45 min after the end of the leg kicking and still had an attenuated HR compared to pre-propranolol baseline (-Δ13 ± 7 bpm).

Table 2. Baseline cardiorespiratory and leg perfusion variables.

	Control		*Propranolol*	
Variable	Males	Females	Males	Females
Cardiorespiratory	n = 8	n = 8	n = 8	n = 8
Hb concentration ($g \cdot dL^{-1}$) *	15.7 ± 1.1	14.8 ± 0.8	15.8 ± 0.8	15.0 ± 0.8
Heart rate (bpm) †	64 ± 8	63 ± 6	59 ± 6	57 ± 4
Mean arterial pressure (mmHg)	86 ± 10	85 ± 8	90 ± 10	84 ± 5
Ventilation ($L \cdot min^{-1}$) †	10.7 ± 2.3	10.0 ± 2.1	9.7 ± 2.0	8.5 ± 1.7
$\dot{V}O_2$ ($L \cdot min^{-1}$) *	0.34 ± 0.06	0.28 ± 0.07	0.37 ± 0.06	0.28 ± 0.06
$\dot{V}CO_2$ ($L \cdot min^{-1}$) *	0.26 ± 0.04	0.22 ± 0.04	0.25 ± 0.04	0.19 ± 0.03
Leg variables				
FA diameter (cm) *	0.93 ± 0.09	0.76 ± 0.10	0.95 ± 0.07	0.76 ± 0.10
Shear rate (s^{-1}) *	80 ± 21	125 ± 35	76 ± 20	119 ± 36
$\dot{Q}_{LEG}$ ($mL \cdot min^{-1}$)	384 ± 136	310 ± 73	377 ± 94	300 ± 58
$n\dot{Q}_{LEG}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	205 ± 68	208 ± 41	203 ± 56	201 ± 34
Est. O_2 del. ($L \cdot min^{-1}$) *	0.08 ± 0.03	0.06 ± 0.01	0.08 ± 0.02	0.06 ± 0.01
LVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	4.6 ± 2.1	3.6 ± 0.8	4.2 ± 1.2	3.6 ± 0.6
nLVC ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot kg^{-1}$)	2.4 ± 1.0	2.5 ± 0.6	2.3 ± 0.6	2.4 ± 0.4

Values are means ± SD. Hb concentration, blood hemoglobin concentration; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, expired carbon dioxide; FA, femoral artery; $\dot{Q}_{LEG}$, leg blood flow; $n\dot{Q}_{LEG}$, normalized leg blood flow; Est. O_2 del., estimated O_2 delivery; LVC, leg vascular conductance; nLVC, normalized leg vascular conductance. Statistical analysis: 2-way ANOVA (sex X propranolol). *****, $P < 0.05$ main effect of sex; **†**, $P < 0.05$ main effect of propranolol.

Baseline

Baseline cardiorespiratory and leg perfusion data can be found in Table 2. No effect of sex or propranolol was found with resting baseline MAP ($P > 0.05$). A main effect of sex was observed

with baseline Hb blood concentrations, $\dot{V}O_2$ and $\dot{V}CO_2$, ΔFA_D, shear rate and estimated O_2 delivery to the leg (all $P < 0.05$). A main effect of propranolol was observed with HR and $\dot{V}_E$ (both $P < 0.05$). Baseline $\dot{Q}_{LEG}$, $n\dot{Q}_{LEG}$, LVC, nLVC were not different between conditions (all $P > 0.05$). No interaction was observed between sex and condition for any baseline variable.

Relative muscle activity

Relative quadriceps activity increased linearly from 11 ± 15% and 20 ± 16% at 3W to 130 ± 43% and 171 ± 88% at 18W for CON and PROP, respectively. Quadriceps muscle activity increased with exercise intensity ($P < 0.01$) and was greater with propranolol ($P < 0.01$), but no interaction ($P = 0.34$) was observed. Maximal relative quadriceps activity was 352 ± 172% and 448 ± 266% for CON and PROP, respectively. Relative hamstring activity was not different from 3W (7 ± 16; 0 ± 10%) to 18W (18 ± 38; 15 ± 43%) for CON and PROP, respectively. No main effect of exercise intensity, propranolol or interaction was observed (all, $P > 0.05$). Maximal relative hamstring activity was 90 ± 112% and 100 ± 126% for CON and PROP, respectively.

Cardiorespiratory variables

Exercising HR and MAP responses during incremental knee extension exercise and relative to peak exercise intensity are found in Figure 3. Cardiorespiratory and local leg perfusion variable response slopes to absolute workloads can be found in Table 3. Main effects of exercise intensity, sex and propranolol were observed for HR ($P < 0.01$) but not MAP ($P > 0.05$). Examination of slope responses found no sex, propranolol or interaction effect with MAP responses (all $P > 0.05$). Heart rate slope responses were not different by sex ($P = 0.25$), but a main effect of propranolol ($P < 0.01$) and an interaction was observed ($P = 0.04$). Further probing of the interaction found both male and female HR slope responses were blunted with propranolol ($P < 0.05$). Relative workload MAP responses had a main effect of exercise intensity (Figure 3D; $P < 0.01$), but no

main effect of propranolol or sex (both $P > 0.05$). Relative workload HR had a significant main effect of exercise intensity and propranolol (both, $P < 0.01$) but not sex ($P = 0.06$). Relative cardiorespiratory and local leg perfusion variables slope values can be found in Table 4. No main effect of sex, propranolol or interaction was found with relative slope MAP responses. No sex difference or interaction was observed with relative HR slope responses, but a main effect of propranolol was observed ($P < 0.01$).

Table 3. Slopes of cardiorespiratory and leg perfusion variables over incremental exercise

	Control		*Propranolol*	
Variable	Male	Female	Male	Female
Cardiorespiratory	n = 8	n = 8	n = 8	n = 8
Heart rate (bpm/W) † #	1.19 ± 0.52	1.60 ± 0.39	0.80 ± 0.43 ‡	0.84 ± 0.27 ‡
(R^2 value)	(0.88 ± 0.13)	(0.95 ± 0.04)	(0.96 ± 0.04)	(0.96 ± 0.03)
MAP (mmHg/W)	0.90 ± 0.29	0.88 ± 0.47	0.80 ± 0.46	0.82 ± 0.58
(R^2 value)	(0.84 ± 0.10)	(0.69 ± 0.29)	(0.79 ± 0.11)	(0.65 ± 0.35)
Ventilation ($L \cdot min^{-1}$/W)	0.95 ± 0.32	0.88 ± 0.18	1.08 ± 0.74	0.84 ± 0.32
(R^2 value)	(0.83 ± 0.13)	(0.93 ± 0.02)	(0.88 ± 0.11)	(0.93 ± 0.05)
$\dot{V}O_2$ ($L \cdot min^{-1}$/W)	0.02 ± 0.005	0.03 ± 0.005	0.03 ± 0.01	0.02 ± 0.005
(R^2 value)	(0.90 ± 0.09)	(0.92 ± 0.07)	(0.91 ± 0.09)	(0.94 ± 0.03)
$\dot{V}CO_2$ ($L \cdot min^{-1}$/W)	0.03 ± 0.009	0.03 ± 0.004	0.03 ± 0.02	0.03 ± 0.009
(R^2 value)	(0.88 ± 0.08)	(0.93 ± 0.02)	(0.90 ± 0.07)	(0.94 ± 0.03)
Leg variables				
ΔFA_D (%/W) * † #	0.07 ± 0.07	0.37 ± 0.32	0.08 ± 0.06	0.23 ± 0.20 ‡
(R^2 value)	(0.50 ± 0.38)	(0.87 ± 0.05)	(0.45 ± 0.24)	(0.65 ± 0.37)
Shear rate (s^{-1}/W)	12.8 ± 3.8	9.4 ± 4.1	15.0 ± 8.7	9.9 ± 3.4
(R^2 value)	(0.95 ± 0.02)	(0.90 ± 0.05)	(0.94 ± 0.04)	(0.88 ± 0.11)
$\dot{Q}_{LEG}$ ($mL \cdot min^{-1}$/W)	57.5 ± 19.9	53.4 ± 15.0	52.2 ± 13.4	48.2 ± 15.3
(R^2 value)	(0.96 ± 0.03)	(0.95 ± 0.02)	(0.97 ± 0.02)	(0.95 ± 0.03)
$n\dot{Q}_{LEG}$ ($mL \cdot min^{-1} \cdot kg^{-1}$/W)	30.7 ± 10.2	36.6 ± 12.4	28.8 ± 10.3	33.0 ± 12.8
(R^2 value)	(0.96 ± 0.03)	(0.95 ± 0.02)	(0.97 ± 0.02)	(0.95 ± 0.03)
Est. O_2 del. ($L \cdot min^{-1}$/W)	0.012 ± 0.004	0.010 ± 0.003	0.011 ± 0.003	0.009 ± 0.003
(R^2 value)	(0.96 ± 0.03)	(0.95 ± 0.02)	(0.97 ± 0.02)	(0.95 ± 0.03)
LVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$/W)	0.42 ± 0.15	0.34 ± 0.17	0.34 ± 0.11	0.32 ± 0.16
(R^2 value)	(0.90 ± 0.05)	(0.75 ± 0.22)	(0.85 ± 0.14)	(0.86 ± 0.17)
nLVC † ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot kg^{-1}$/W)	0.23 ± 0.11	0.27 ± 0.10	0.18 ± 0.10	0.19 ± 0.10
(R^2 value)	(0.88 ± 0.09)	(0.87 ± 0.04)	(0.80 ± 0.26)	(0.74 ± 0.19)

Values are means ± SD. MAP, mean arterial pressure; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, expired carbon dioxide; FA, femoral artery; $\dot{Q}_{LEG}$, leg blood flow; $n\dot{Q}_{LEG}$, normalized leg blood flow; Est. O_2 del, estimated O_2 delivery; LVC, leg vascular conductance; nLVC, normalized leg vascular conductance. Statistical analysis: 2-way ANOVA (sex X propranolol). *$P < 0.05$ main effect of sex; †$P < 0.05$ main effect of propranolol; #$P < 0.05$ sex x propranolol interaction; ‡$P < 0.05$ slope response significantly different than control.

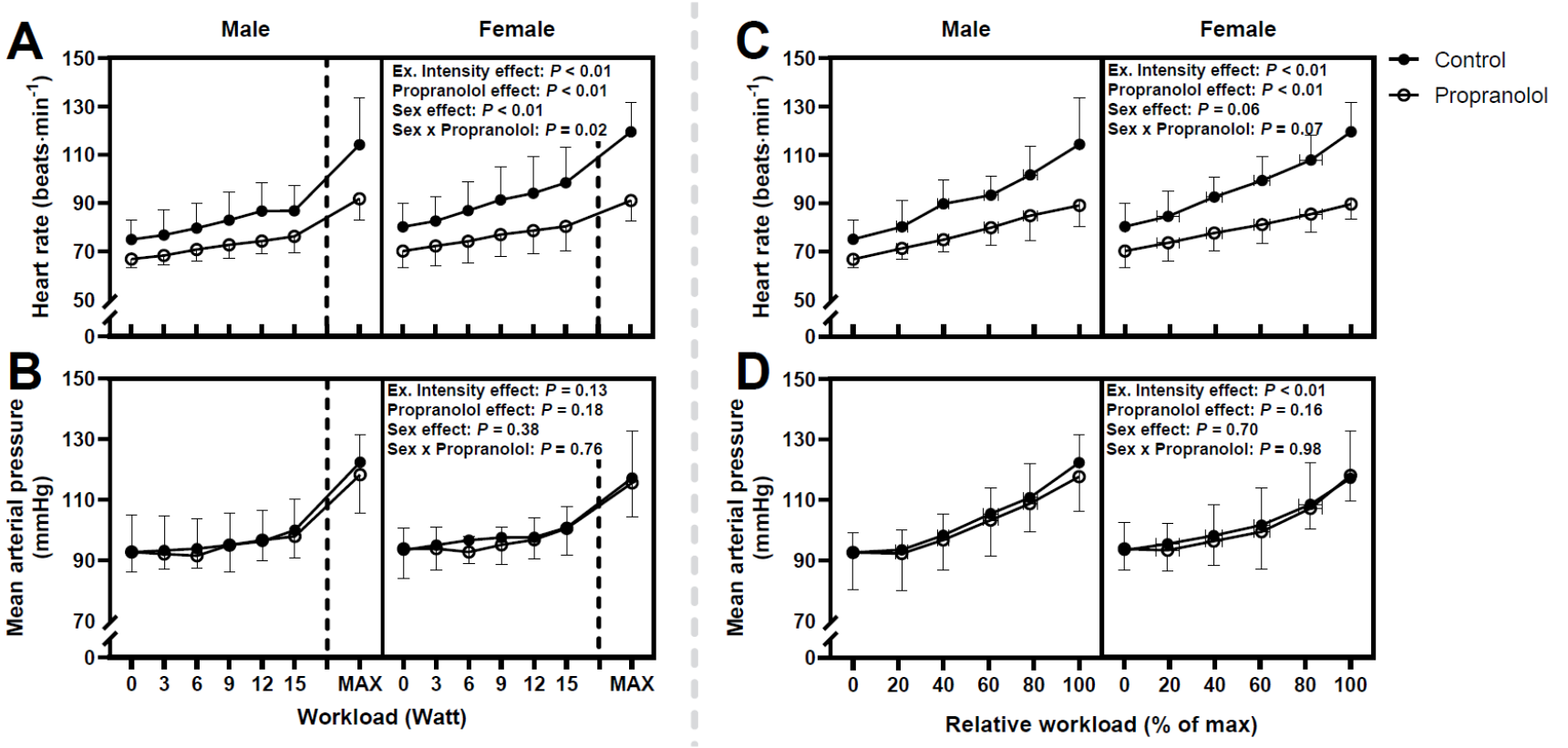


Figure 3. Heart rate and mean arterial responses to incremental knee extension exercise

Absolute heart rate (**A**) and mean arterial pressure (**B**) responses to incremental single-leg knee extension exercise in males (n = 8) and females (n = 8) for both conditions. Relative heart rate (**C**) and mean arterial pressure (**D**) responses to exercise relative to max exercise intensity in males (n = 8) and females (n = 8) for control condition. For propranolol condition, male n = 8 and female n = 8 up to 80% and at 100% male n = 7 and female n = 5. Control and propranolol conditions denoted by closed and open circles, respectively. For control condition, MAX workload was 35.6 ± 13.2 W in males and 25.4 ± 10.2 W in females. For propranolol condition, MAX workload was 35.3 ± 13.5 W in males and 25.4 ± 10.7 W in females. Statistical analysis: 3-way ANOVA (sex X propranolol X exercise intensity).

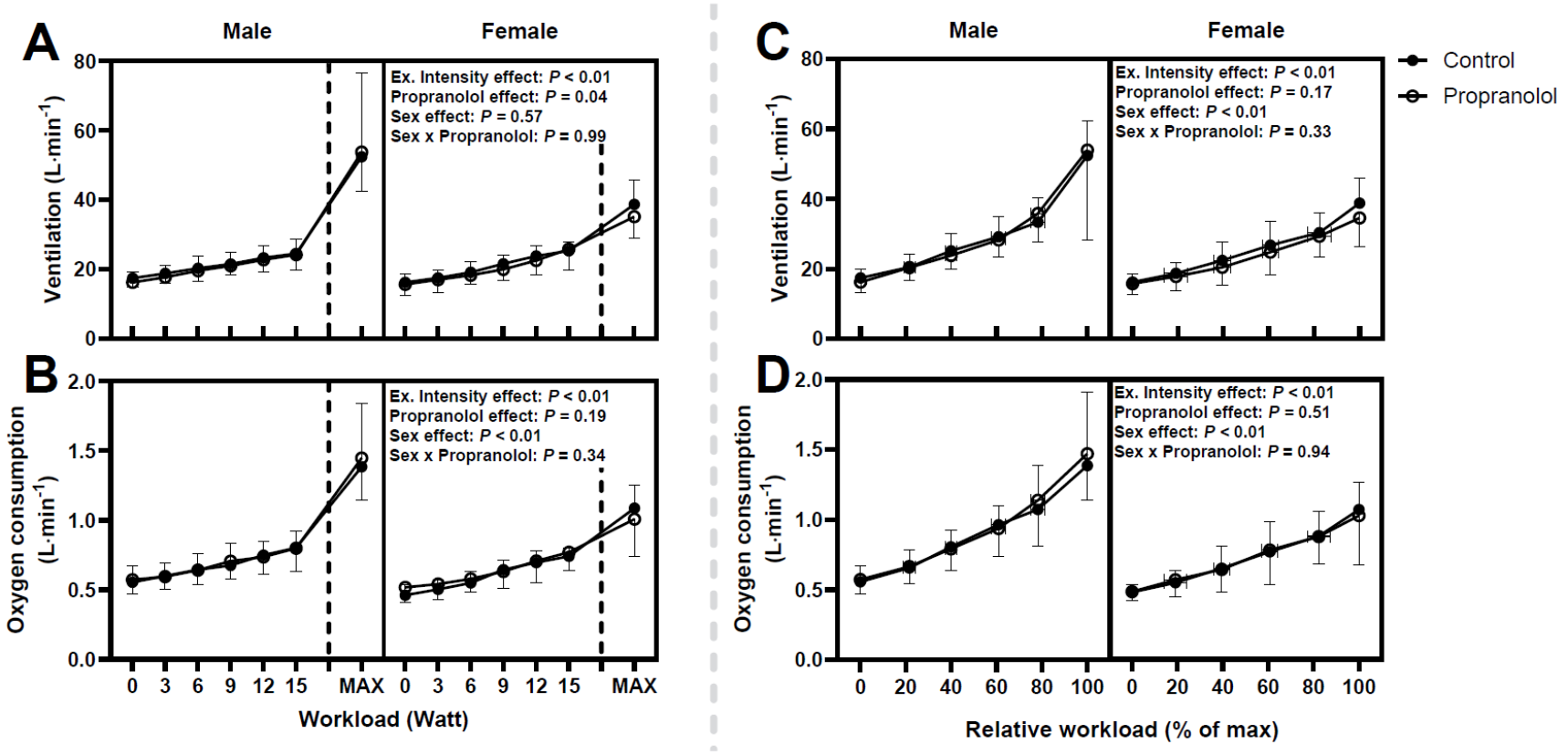


Figure 4. Ventilation and oxygen consumption responses to incremental knee extension exercise

Absolute ventilation (**A**) and oxygen consumption (**B**) responses to incremental single-leg knee extension exercise in males (n = 8) and females (n = 8) for both conditions. Relative ventilation (**C**) and oxygen consumption (**D**) responses to exercise relative to max exercise intensity in males (n = 8) and females (n = 8) for control condition. For propranolol condition, male n = 8 and female n = 8 up to 80% and at 100% male n = 7 and female n = 5. Control and propranolol conditions denoted by closed and open circles, respectively. For control condition, MAX workload was 35.6 ± 13.2 W in males and 25.4 ± 10.2 W in females. For propranolol condition, MAX workload was 35.3 ± 13.5 W in males and 25.4 ± 10.7 W in females. Statistical analysis: 3-way ANOVA (sex X propranolol X exercise intensity).

Ventilation and $\dot{V}O_2$ responses to incremental knee extension exercise and relative to peak workload can be found in Figure 4. For $\dot{V}_E$ and $\dot{V}O_2$, a main effect of exercise intensity was observed (both, $P < 0.01$) but only $\dot{V}_E$ had a main effect of propranolol ($P < 0.05$). A main effect of sex was observed with $\dot{V}O_2$ ($P < 0.01$) but not ventilation ($P = 0.56$). Slope responses of $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ to absolute workloads found no sex, propranolol or interaction effects (both, $P > 0.16$). Relative workload $\dot{V}_E$ and $\dot{V}O_2$ responses had significant exercise intensity and sex effects (both, $P < 0.01$), but not propranolol (both, $P > 0.05$). Relative workload $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ slope responses were not different by sex or propranolol, and no interactions were observed (all, $P > 0.05$).

Table 4. Slopes of cardiorespiratory and leg perfusion variables over relative exercise intensity.

	Control		*Propranolol*	
Variable	Male	Female	Male	Female
Cardiorespiratory	n = 8	n = 8	n = 8	n = 8
Heart rate (bpm/%) †	0.33 ± 0.15	0.34 ± 0.13	0.23 ± 0.10	0.19 ± 0.07
(R^2 value)	(0.86 ± 0.19)	(0.97 ± 0.02)	(0.98 ± 0.02)	(0.97 ± 0.03)
MAP (mmHg/%)	0.24 ± 0.10	0.18 ± 0.16	0.22 ± 0.13	0.17 ± 0.10
(R^2 value)	(0.88 ± 0.06)	(0.69 ± 0.32)	(0.76 ± 0.19)	(0.64 ± 0.30)
Ventilation ($L \cdot min^{-1}$/%)	0.20 ± 0.10	0.18 ± 0.07	0.24 ± 0.09	0.17 ± 0.07
(R^2 value)	(0.80 ± 0.32)	(0.94 ± 0.05)	(0.93 ± 0.08)	(0.94 ± 0.04)
$\dot{V}O_2$ ($mL \cdot min^{-1}$/%)	6.76 ± 3.46	4.99 ± 2.21	7.04 ± 2.73	4.78 ± 1.86
(R^2 value)	(0.84 ± 0.25)	(0.93 ± 0.03)	(0.92 ± 0.07)	(0.93 ± 0.05)
$\dot{V}CO_2$ ($mL \cdot min^{-1}$/%)	7.12 ± 2.82	5.24 ± 1.82	8.10 ± 2.61	5.81 ± 2.00
(R^2 value)	(0.90 ± 0.12)	(0.94 ± 0.04)	(0.95 ± 0.04)	(0.95 ± 0.04)
Leg variables				
ΔFA_D (%/%)*†	0.03 ± 0.02	0.09 ± 0.06	0.02 ± 0.02	0.06 ± 0.04
(R^2 value)	(0.70 ± 0.27)	(0.84 ± 0.15)	(0.49 ± 0.23)	(0.72 ± 0.29)
Shear rate (s^{-1}/%)	3.5 ± 1.6	2.5 ± 0.8	3.1 ± 0.9	2.9 ± 0.9
(R^2 value)	(0.95 ± 0.05)	(0.89 ± 0.07)	(0.97 ± 0.01)	(0.89 ± 0.13)
$\dot{Q}_{LEG}$ ($mL \cdot min^{-1}$/%)	18.41 ± 5.13	11.75 ± 3.94	16.51 ± 3.31	11.15 ± 5.16
(R^2 value)	(0.95 ± 0.06)	(0.94 ± 0.04)	(0.97 ± 0.03)	(0.92 ± 0.08)
$n\dot{Q}_{LEG}$ * ($mL \cdot min^{-1} \cdot kg^{-1}$/%)	9.77 ± 2.40	7.90 ± 2.59	8.88 ± 1.98	7.50 ± 3.47
(R^2 value)	(0.95 ± 0.06)	(0.94 ± 0.04)	(0.97 ± 0.03)	(0.92 ± 0.08)
Est. O_2 del. ($L \cdot min^{-1}$/%) *	0.004 ± 0.001	0.002 ± 0.001	0.003 ± 0.001	0.002 ± 0.001
(R^2 value)	(0.95 ± 0.06)	(0.94 ± 0.04)	(0.97 ± 0.03)	(0.92 ± 0.08)
LVC * ($mL \cdot min^{-1} \cdot mmHg^{-1}$/%)	0.13 ± 0.05	0.08 ± 0.02	0.11 ± 0.04	0.08 ± 0.04
(R^2 value)	(0.85 ± 0.16)	(0.88 ± 0.12)	(0.85 ± 0.21)	(0.83 ± 0.17)
nLVC ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot kg^{-1}$/%)	0.07 ± 0.03	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.03
(R^2 value)	(0.85 ± 0.16)	(0.88 ± 0.12)	(0.85 ± 0.21)	(0.83 ± 0.17)

Values are means ± SD. MAP, mean arterial pressure; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, expired carbon dioxide; FA, femoral artery; $\dot{Q}_{LEG}$, leg blood flow; $n\dot{Q}_{LEG}$, normalized leg blood flow; Est. O_2 del, estimated O_2 delivery; LVC, leg vascular conductance; nLVC, normalized leg vascular conductance. Statistical analysis: 2-way ANOVA (sex X propranolol). *$P < 0.05$ main effect of sex; †$P < 0.05$ main effect of propranolol; #$P < 0.05$ sex x propranolol interaction; ‡$P < 0.05$ slope response significantly different than control.

Leg hyperemia response.

The percent change in FA diameter during incremental exercise and relative to peak workload can be observed in Figure 5. A main effect of propranolol and sex were observed for ΔFA_D (both, $P < 0.05$), but not with exercise intensity ($P = 0.22$). However, a main effect of sex, propranolol and an interaction was observed for the ΔFA_D slope responses (all, $P < 0.05$). Further analysis found females to have a blunted vasodilatory response with propranolol ($P < 0.01$), while males were not different from control ($P = 0.99$). When ΔFA_D was adjusted to peak workloads, a main effect of exercise intensity, sex and propranolol was found (all, $P < 0.01$). Slope responses of ΔFA_D scaled to peak workload showed significant propranolol and sex effects (both $P < 0.05$), but no interaction ($P = 0.18$). No effect of sex, propranolol or interaction was observed for shear rate slope responses at absolute and relative workloads ($P > 0.05$).

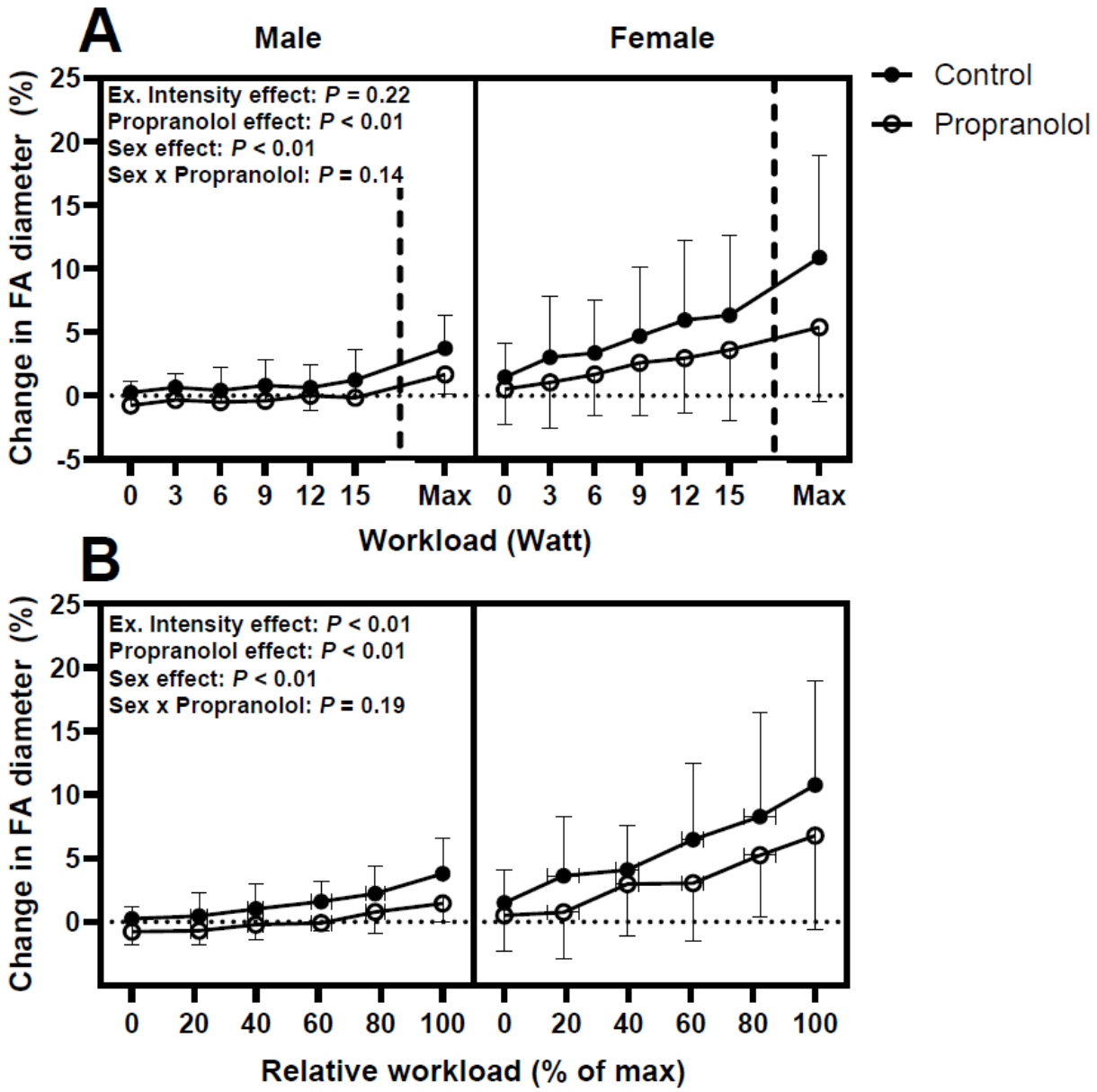


Figure 5. Change in femoral artery diameter from baseline during incremental knee extension exercise

Percent change in femoral artery diameter from baseline during single-leg knee extension exercise in males (n = 8) and females (n = 8) for both conditions (**A**). Percent change in femoral artery diameter from baseline during exercise relative to maximal exercise intensity (**B**) in males (n = 8) and females (n = 8) for control condition. For propranolol condition, male n = 8 and female n = 8 up to 80% and at 100% male n = 7 and female n = 5. Control and propranolol conditions denoted by closed and open circles, respectively. For control condition, MAX workload was 35.6 ± 13.2 W in males and 25.4 ± 10.2 W in females. For propranolol condition, MAX workload was 35.3 ± 13.5 W in males and 25.4 ± 10.7 W in females. Statistical analysis: 3-way ANOVA (sex X propranolol X exercise intensity).

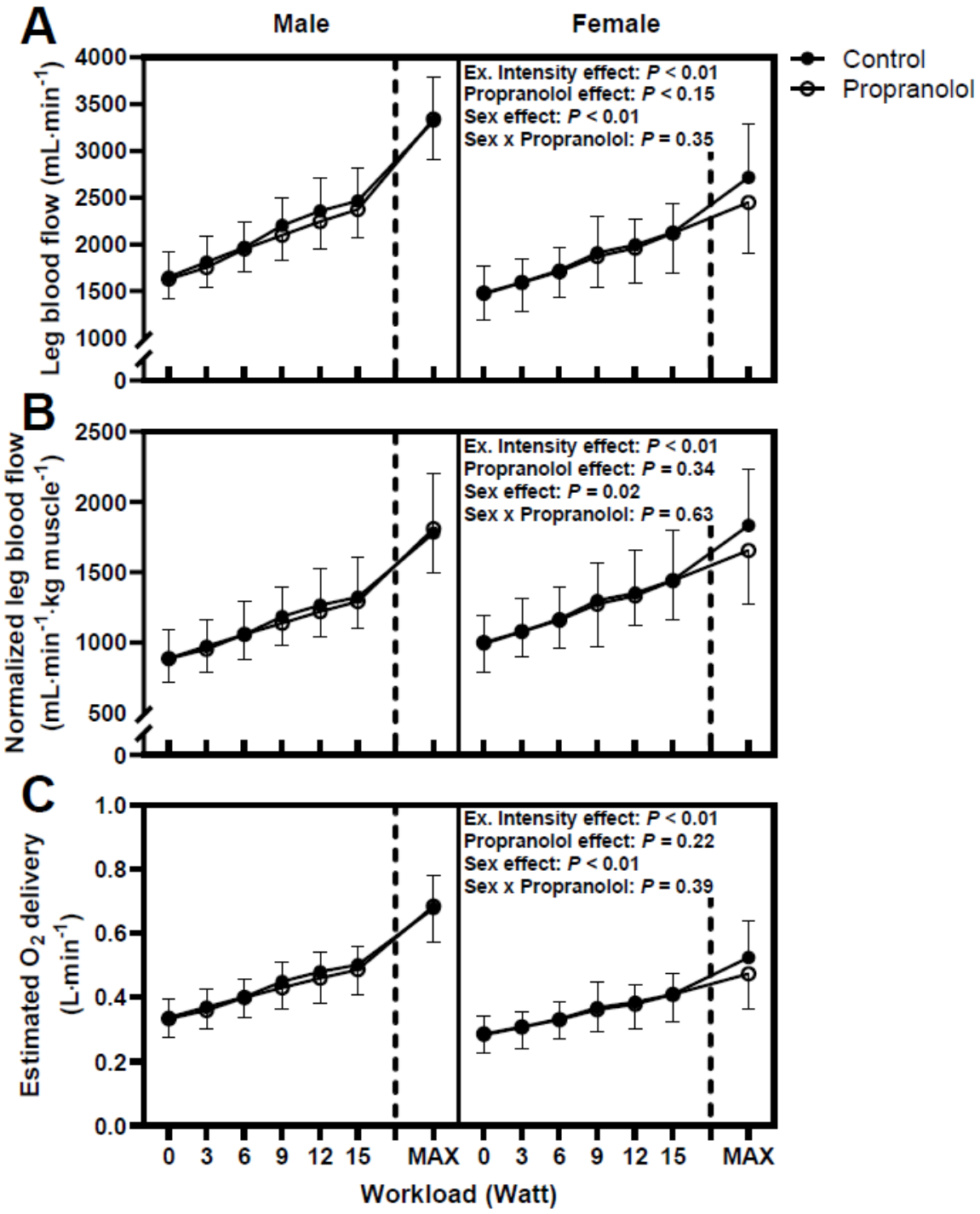


Figure 6. Leg blood flow responses to absolute increases in workload during incremental knee extension exercise

Leg blood flow (**A**), normalized leg blood flow (**B**), and estimated O_2 delivery (**C**) responses to single-leg knee extension exercise in males (n = 8) and females (n = 8) for both conditions. Control and propranolol conditions are denoted by closed and open circles, respectively. For control condition, MAX workload was 35.6 ± 13.2 W in males and 25.4 ± 10.2 W in females. For propranolol condition, MAX workload was 35.3 ± 13.5 W in males and 25.4 ± 10.7 W in females. Statistical analysis: 3-way ANOVA (sex X propranolol X exercise intensity).

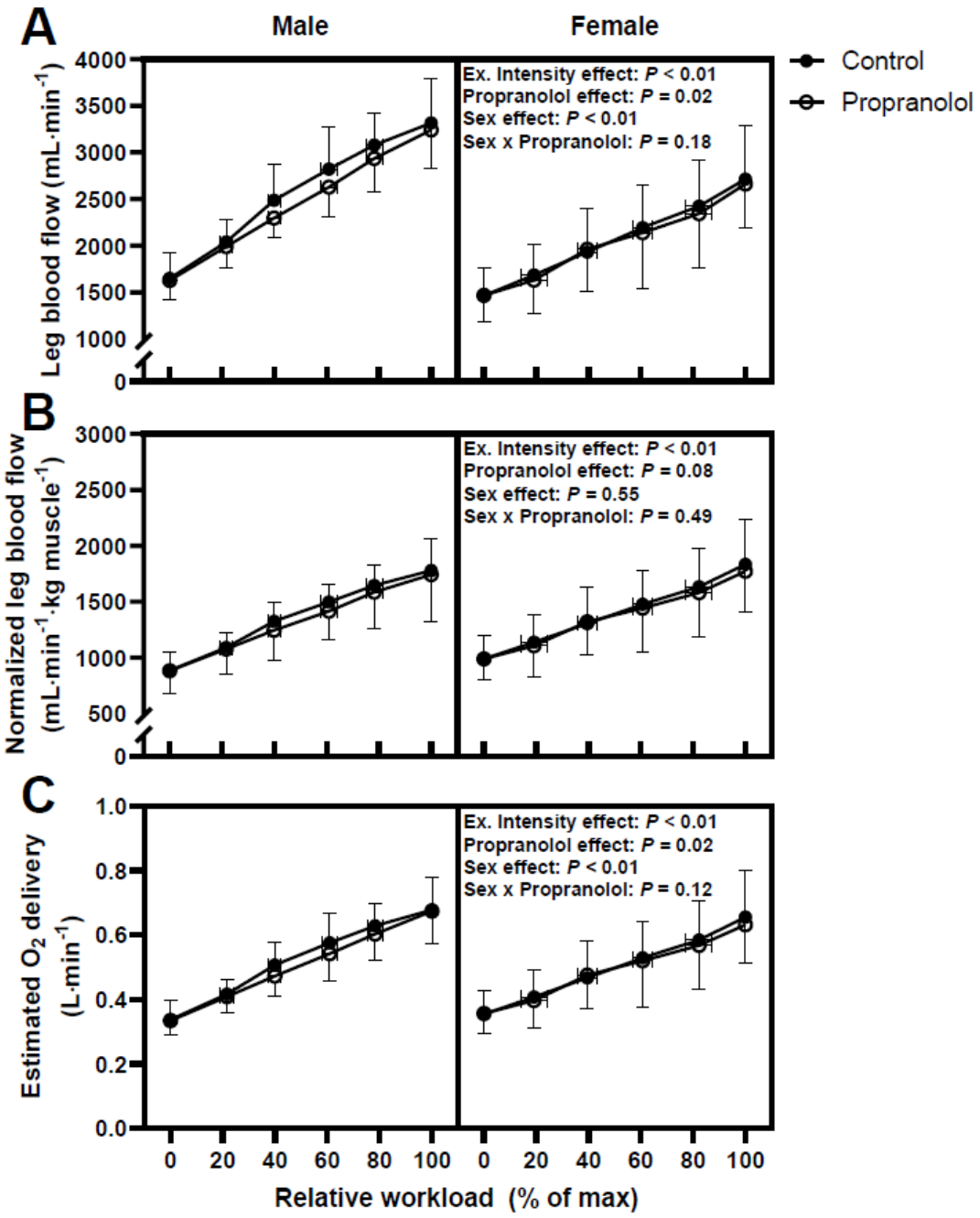


Figure 7. Leg blood flow responses to relative increases in workload during incremental knee extension exercise

Leg blood flow (**A**), normalized leg blood flow (**B**), and estimated O_2 delivery (**C**) responses to single-leg knee extension exercise relative to maximal exercise intensity in males (n = 8) and females (n = 8) for control condition. For propranolol condition, male n = 8 and female n = 8 up to 80% and at 100% male n = 7 and female n = 5. Control and propranolol conditions are denoted by closed and open circles, respectively. For control condition, MAX workload was 35.6 ± 13.2 W in males and 25.4 ± 10.2 W in females. For propranolol condition, MAX workload was 35.3 ± 13.5 W in males and 25.4 ± 10.7 W in females. Statistical analysis: 3-way ANOVA (sex X propranolol X exercise intensity).

Leg blood flow, $n\dot{Q}_{LEG}$ and estimated O_2 delivery to the leg in response to incremental knee extension exercise can be found in Figure 6. Main effects of exercise intensity and sex (all, $P < 0.05$) but not propranolol (all, $P > 0.15$) was observed for $\dot{Q}_{LEG}$, $n\dot{Q}_{LEG}$ and estimated O_2 delivery. However, no main effects of propranolol, sex or an interaction was found for $\dot{Q}_{LEG}$, $n\dot{Q}_{LEG}$ and estimated O_2 delivery slope responses (Table 3; all, $P > 0.21$). Relative $\dot{Q}_{LEG}$, $n\dot{Q}_{LEG}$ and estimated leg O_2 delivery responses are shown in Figure 7. A main effect of exercise intensity was observed for relative workload $\dot{Q}_{LEG}$, $n\dot{Q}_{LEG}$ and estimated O_2 delivery (all $P < 0.05$). A main effect of propranolol was found in $\dot{Q}_{LEG}$ and estimated O_2 delivery to the leg (both, $P < 0.05$), but not $n\dot{Q}_{LEG}$ ($P = 0.08$). A main effect of sex was also observed for relative workload $\dot{Q}_{LEG}$ and estimated O_2 delivery (both $P < 0.01$), but not $n\dot{Q}_{LEG}$ ($P = 0.64$). A main effect of sex was observed with relative workload $\dot{Q}_{LEG}$ and leg O_2 delivery slope responses (both $P < 0.02$), but not $n\dot{Q}_{LEG}$ ($P = 0.20$). No main effect of propranolol or interaction was observed for relative workload $\dot{Q}_{LEG}$, $n\dot{Q}_{LEG}$ and estimated leg O_2 delivery slope responses (all $P > 0.17$).

Leg vascular conductance and nLVC responses to incremental exercise and relative to peak exercise intensity are shown in Figure 8. A main effect of exercise intensity (both, $P < 0.05$), but not propranolol (both, $P > 0.05$) was observed for both LVC and nLVC. A main effect of sex was observed with LVC ($P < 0.01$), but not nLVC ($P = 0.07$). No effect of sex, propranolol or interaction was observed with LVC slope responses (Table 3; all, $P > 0.35$). However, a significant propranolol effect was observed with nLVC slope response ($P = 0.02$), but no sex effect or interaction was found (both $P > 0.66$). Relative to peak workload, a main effect of exercise (both $P < 0.01$) but not propranolol (both, $P > 0.22$) was present for LVC and nLVC. A main effect of sex was present for LVC ($P < 0.01$) but was eliminated after normalizing for muscle mass ($P = 0.51$). A significant sex effect was found for relative workload LVC slope responses (Table 4; $P =$

0.02), but no effect of propranolol or interaction was observed (both $P > 0.51$). Relative nLVC responses were not different with sex or propranolol and no interaction was observed (all $P > 0.31$).

Table 5. Cardiorespiratory and leg perfusion variables during peak exercise intensity.

	Control		*Propranolol*	
Variable	Males	Females	Males	Females
Cardiorespiratory	n = 8	n = 8	n = 8	n = 8
Peak exercise intensity (W)	35.6 ± 13.2	25.4 ± 10.2	35.3 ± 13.5	25.4 ± 10.7
Heart rate (bpm) †	114 ± 19	119 ± 12	92 ± 9	91 ± 8
Mean arterial pressure (mmHg)	122 ± 9	117 ± 16	118 ± 13	116 ± 11
Ventilation ($L \cdot min^{-1}$) *	52.4 ± 9.9	38.7 ± 7.2	53.8 ± 22.7	35.1 ± 6.2
$\dot{V}O_2$ ($L \cdot min^{-1}$) *	1.39 ± 0.24	1.09 ± 0.17	1.45 ± 0.39	1.01 ± 0.26
$\dot{V}CO_2$ ($L \cdot min^{-1}$) *	1.48 ± 0.23	1.06 ± 0.11	1.54 ± 0.52	1.01 ± 0.19
Leg variables				
ΔFA_D (%) * †	3.7 ± 2.6	10.9 ± 8.1	1.7 ± 1.5	5.4 ± 5.9
Shear rate (s^{-1})	644 ± 190	794 ± 95	655 ± 168	803 ± 176
$\dot{Q}_{LEG}$ ($mL \cdot min^{-1}$) *	3316 ± 469	2715 ± 575	3339 ± 426	2447 ± 540
$n\dot{Q}_{LEG}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	1778 ± 285	1833 ± 406	1809 ± 392	1654 ± 387
Est. O_2 del. ($L \cdot min^{-1}$) *	0.68 ± 0.10	0.52 ± 0.11	0.68 ± 0.11	0.47 ± 0.11
LVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$) *	27.3 ± 4.5	23.4 ± 5.2	28.6 ± 4.9	21.2 ± 4.0
nLVC ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot kg^{-1}$)	14.7 ± 2.9	15.9 ± 4.5	15.5 ± 3.8	14.4 ± 3.3

Values are means ± SD. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, expired carbon dioxide; %Δ FA diameter, percent change in femoral artery from baseline; $\dot{Q}_{LEG}$, leg blood flow; $n\dot{Q}_{LEG}$, normalized leg blood flow; Est. O_2 del., estimated O_2 delivery; LVC, leg vascular conductance; nLVC, normalized leg vascular conductance. Statistical analysis: 2-way ANOVA (sex X propranolol). *$P < 0.05$ main effect of sex; †$P < 0.05$ main effect of propranolol.

Peak exercise intensity. Peak cardiorespiratory and leg perfusion variables are shown in Table 5. No effect of sex or propranolol was found with MAP, shear rate, $n\dot{Q}_{LEG}$ and nLVC at peak exercise (all $P > 0.05$). A main effect of sex was observed with $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$, ΔFA_D, $\dot{Q}_{LEG}$, estimated O_2 delivery and LVC (all $P < 0.05$). A main effect of propranolol was observed with HR and ΔFA_D (both $P<0.05$). No interaction was observed between sex and condition for any peak cardiorespiratory or leg perfusion variable (all $P > 0.05$).

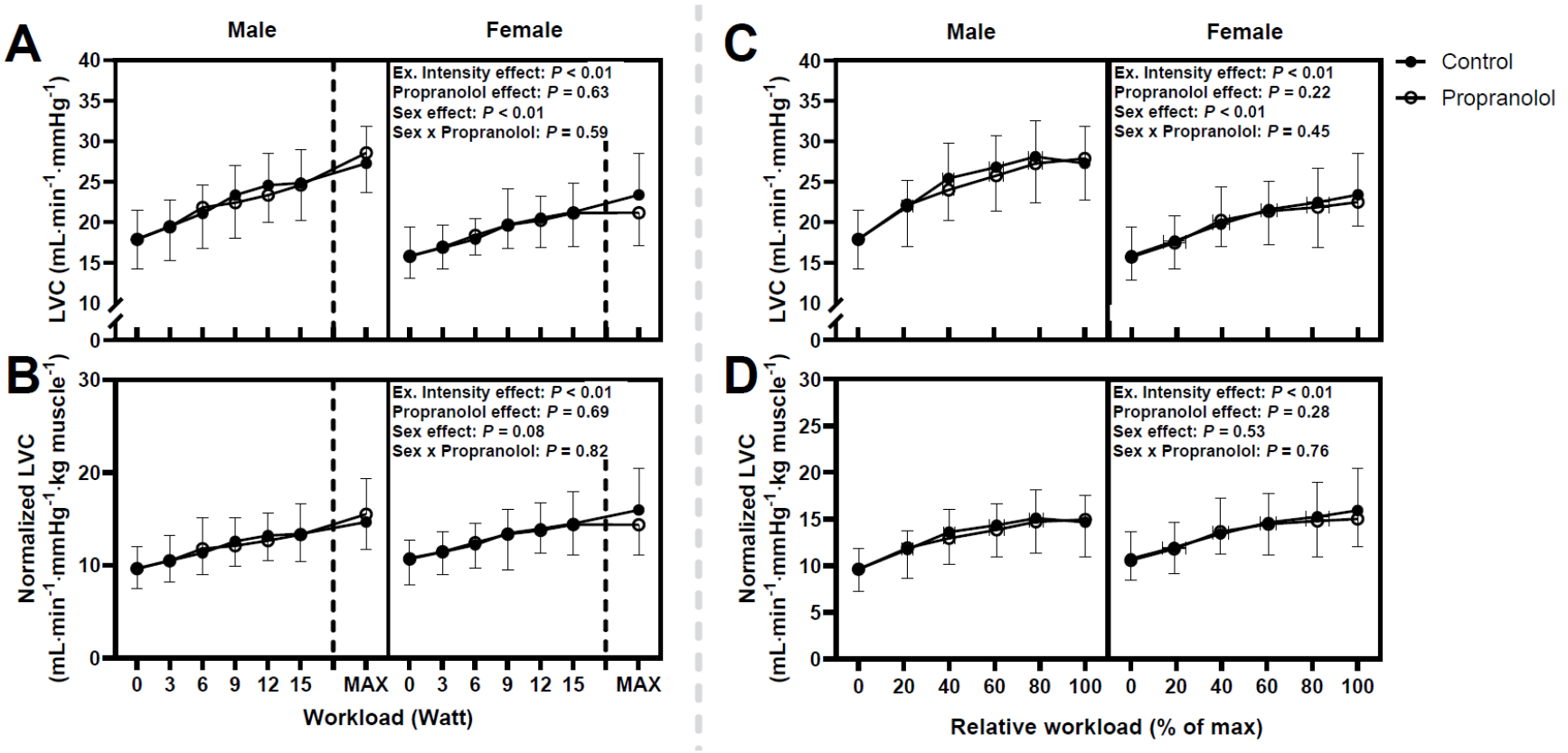


Figure 8. Leg vascular conductance responses to absolute and relative increases in workload during incremental knee extension exercise.

Absolute leg vascular conductance (**A**) and normalized leg vascular conductance (**B**) responses to incremental single-leg knee extension exercise in males (n = 8) and females (n = 8) for both conditions. Relative leg vascular conductance (**C**) and normalized leg vascular conductance (**D**) responses to exercise relative to max exercise intensity in males (n = 8) and females (n = 8) for control condition. For propranolol condition, male n = 8 and female n = 8 up to 80% and at 100% male n = 7 and female n = 5. Control and propranolol conditions are denoted by closed and open circles, respectively. For control condition, MAX workload was 35.6 ± 13.2 W in males and 25.4 ± 10.2 W in females. For propranolol condition, MAX workload was 35.3 ± 13.5 W in males and 25.4 ± 10.7 W in females. LVC, Leg vascular conductance. Statistical analysis: 3-way ANOVA (sex X propranolol X exercise intensity).

3.4 Discussion

We sought to determine the role of β-ARs in mediating exercise hyperemia in males and females during incremental knee extension exercise to failure. The major findings from the study are two-fold. First, there was no difference in the slope response to incremental exercise for $\dot{Q}_{LEG}$, LVC or O_2 delivery to the leg to workload with or without β-AR blockade between males and females. The lack of sex differences in the hyperemia slope response remained even after normalizing for muscle mass and peak workloads. We interpret these findings to suggest that exercise hyperemia is not principally dependent on β-AR as oxygen delivery to the exercising skeletal muscles was preserved in both males and females. Second, we observed greater FA dilation in females during that was blunted to a greater extent with propranolol compared to males. This finding suggests local β_2-ARs may be involved in the dilation of large conduit arteries during exercise, and that females rely more on β-AR-mediated dilation of the FA artery to mediate increases in exercise hyperemia. Overall, our study supports the notion of numerous mechanisms mediating exercise hyperemia in both sexes, but that β_2-AR in blood vessels play a greater role in the control of vascular tone in females.

Sex differences in exercise hyperemia

When examining absolute workloads, the present study found that males had a greater absolute $\dot{Q}_{LEG}$ and LVC response at a given workload (Figures 6A, 8A). However, after normalizing for individual quadriceps muscle mass, $n\dot{Q}_{LEG}$ and nLVC were greater in females (Figures 6B, 8B). These results differed from others that found females had greater absolute $\dot{Q}_{LEG}$ and LVC at a given workload both before and after normalizing quadriceps muscle mass (10). The discrepancy in these findings can be explained by sex differences in quadriceps muscle mass and hemoglobin content. Indeed, males have a greater absolute $\dot{Q}_{LEG}$ and LVC response due to greater

quadriceps muscle mass thus requiring higher absolute O_2 demand (Table 1). Equally unsurprising was the greater hyperemia in females after adjusting for quadriceps muscle mass since a lower hemoglobin content in females necessitates greater blood flows to deliver the same amount of O_2. In other studies investigating sex differences in hyperemia responses to handgrip exercise, evidence exists both for (183, 185) or against (252, 260) greater blood flow and vascular conductance responses in females. However, it is difficult to interpret data from these studies as blood Hb concentration was not reported, and an estimate of CaO_2 was not determined. Since O_2 delivery is the primary objective of exercise hyperemia (1, 2), possible differences in blood O_2 carrying capacity, secondary to differences in population Hb concentrations, could explain the equivocal findings. Thus, it remains difficult to draw clear conclusions whether sex differences in exercise hyperemia are present when only absolute blood flow and vascular conductance are reported.

To further examine sex differences in the exercise hyperemia response without the confounding influence of baseline sex differences in anthropometrics, we examined the individual slope responses of $\dot{Q}_{LEG}$, LVC, $n\dot{Q}_{LEG}$ and nLVC to increases in absolute and relative workloads. We did not find any sex differences in $\dot{Q}_{LEG}$ and LVC or $n\dot{Q}_{LEG}$ and nLVC in slope responses to absolute workload (Table 3). However, when slope responses to relative workloads were examined, males had steeper slope responses to $\dot{Q}_{LEG}$ and LVC (Table 4). The greater response in males can be explained by males achieving greater absolute workloads, thus requiring higher absolute $\dot{Q}_{LEG}$ and LVC responses, leading to steeper slope responses at relative workloads. However, after normalizing to quadriceps muscle mass, $n\dot{Q}_{LEG}$ and nLVC slope responses to relative workloads was not different by sex (Table 4). Our findings suggest that males and females are not different in their ability to perfuse active quadriceps muscle during exercise as similar slope

responses were observed after adjusting for sex differences in quadriceps muscle mass and maximal workloads.

The importance of sex differences in Hb concentration and resting MAP values are highlighted when we compare results from present study to prior work. In the only other study to examine sex differences in $\dot{Q}_{LEG,}$ LVC, $n\dot{Q}_{LEG}$ and nLVC slope responses in females were significantly steeper for both absolute and relative workloads (10). In both studies, Hb concentrations were greater in males; however, females in the present study were only minimally lower than males (control Δ0.9 g/dL; propranolol Δ0.8 g/dL). In contrast, Hb concentrations were substantially lower in females relative to males in the previous study (12.8 ± 0.2 vs. 15.0 ± 0.2 g/dL, respectively) (10). Thus, to ensure sufficient O_2 delivery to active tissues, females achieved greater increases in blood flow relative to males. Indeed, previous studies manipulating Hb blood concentrations during double leg knee extension exercise found $\dot{Q}_{LEG}$ responses to be associated with CaO_2 (261, 262). Differences in LVC responses between the present study results and previous work could also be explained by the differences in resting MAP. Females in the Parker *et al.* study had significantly lower MAP pressures at rest and during exercise relative to males. For example, resting MAP was ~73 mmHg vs. ~85 mmHg; and at 60% relative exercise intensity: ~83 mmHg vs. ~98 mmHg for females and males, respectively in the previous study (10). In contrast, no effect of sex was observed when comparing relative MAP responses at rest or during exercise at similar relative workloads in the present study (Table 2, Figure 3). Together, the contrast in MAP response in combination with the increased $\dot{Q}_{LEG}$ response in females due to lower Hb blood concentrations could explain the divergence in LVC and nLVC between present and past results.

Sex differences in FA dilation response

Dilation of feed arteries is an important regulatory mechanism during exercise that facilitates the increase in blood flow to high-demand tissues. Findings from our study agree with previous work where an increase in FA diameter was observed during knee extension exercise (10). But this is not a universal finding, as others have reported no differences in FA artery diameter from baseline to exercise (91, 263). The discrepancy in FA dilation may be explained by differences in study population as only males were previously tested (91, 263). Indeed, females were found to have a significantly greater FA artery dilation response to incremental exercise (Figure 5). When examining absolute workloads, females had a robust intensity-dependent increase in FA diameter, while males had a significantly lower increase that was poorly related to exercise intensity (Table 3).

The mechanism(s) responsible for this significant sex effect during exercise remains unclear, and we postulate two potential mechanisms. First, due to smaller artery dimensions in females, greater flow velocities are necessitated to deliver similar levels of blood flow. Therefore, one possibility is the greater amount of mechanical shear female arteries experience relative to their male counterparts. Work examining sex differences in brachial artery flow-mediated dilation response to sustained shear stress during handgrip exercise found that young females, but not young males, maintained their ability to vasodilate following 30 minutes of oscillatory shear stress (179). In the present study, shear rate was significantly greater in females at baseline and tended to be greater at peak exercise ($P = 0.07$) (Tables 2, 5). However, the absence of sex differences in shear rate slope responses to absolute and relative workloads (Tables 3, 4) suggests that another mechanism could be involved in mediating the greater FA dilation in females.

Second, β-AR blockade significantly blunted the ΔFA_D slope response to absolute increases in workload in females, but not males. These results agree with previous work in rodents that found enhanced arteriole dilation in females but not males (264). Other work in humans also suggests young females have enhanced β-AR vasodilator responsiveness (8, 181, 265). Young females, but not young males, had a reduced vasoconstrictor response to alpha-adrenergic-mediated vasoconstriction at rest that was increased in females during β-AR blockade with propranolol (8). However, when ΔFA_D slope responses were examined relative to exercise intensities in the present study, the sex difference in the propranolol effect was eliminated (Table 4). Indeed, β-AR blockade blunted male and female FA dilation at peak exercise by a relatively similar extent compared to control (~46% and ~50%, respectively) (Table 5). This finding suggests β-AR function is not different by sex, but rather young females have a greater presence of β_2-ARs in their peripheral vasculature. Altogether, these results suggest a role for local β_2-ARs in mediating the dilation of FA diameters during exercise, and that females appear to rely more on conduit artery dilation to increase exercise hyperemia. Future studies should be performed to examine the possibility that β-AR-mediated vasodilation interacts with mechanical shear-mediated dilation of large conduit arteries as both vasodilate via the nitric oxide pathway (266, 267).

Role of beta receptors in the hyperemia response

Increased estrogen levels in females have been associated with the upregulation or sensitization, of β_2-ARs in the peripheral vasculature (265). Parker and colleagues provided evidence for an estrogen-related mechanism in mediating sex differences in the exercise hyperemia response when they found older post-menopausal females and young males to have blunted $\dot{Q}_{LEG}$ and LVC responses that remained after adjusting for individual peak exercise intensity and muscle mass (11). Since sex differences in resting hemodynamics were previously linked to differences

in β_2-AR vasodilator responsiveness (8), this mechanism might also explain sex differences in exercise hyperemia. Present study results refute this notion as (1) there were no sex differences in $\dot{Q}_{LEG}$ or LVC slope responses during the control condition and (2) complete β-AR blockade with propranolol did not change the $\dot{Q}_{LEG}$ or LVC slope response to exercise in females. There is evidence that β-ARs play a diminishing role in the exercise hyperemia response as increases in exercise intensity decreased β-AR mediated increases in vasodilation (233, 254). During hypoxic submaximal rhythmic handgrip at 10% of max effort, the increase in forearm vascular conductance was almost entirely accounted for by β_2-AR vasodilation; however, β_2-ARs had a significantly reduced role in mediating the increase in forearm vascular conductance during rhythmic handgrip at 20% of max effort (233). Given that exercise intensity appears to be a determining factor in β_2-AR-mediated vasodilation, it might be that the single-leg knee extension exercise model recruits a magnitude of muscle mass that is too large to investigate the role of β_2-ARs in the exercise hyperemia response. In addition, others have found no sex differences in β_2-AR sensitivities during local isoproterenol infusion, but this study was done at rest (268).

Implications for the exercise hyperemia response

Despite observed differences in vascular reactivity between males and females (Figure 5), our study found no sex-based differences in the overall exercise hyperemia response. Our finding of enhanced FA dilation in females aligns with the idea that β_2-ARs mediated sex differences in vascular control, as complete β-AR blockade led to a significantly greater blunting of FA diameter in females, but not males at absolute workloads (Table 3). However, our main finding that slope responses of $\dot{Q}_{LEG}$, LVC and estimated leg O_2 delivery (Tables 3, 4) were not different between sexes and were preserved after β_2-AR blockade suggests sex differences in β_2-AR vasodilator responsiveness primarily observed at rest are insignificant during the cardiorespiratory response

to exercise. Instead, our results support the view that compensatory hyperemia mechanisms are activated during exercise to ensure adequate O_2 delivery, regardless of differences in vascular reactivity between males and females. This is consistent with previous research, which found no change in exercise hyperemia responses when targeting single vasodilator pathways, and only observed significant effects when multiple pathways were inhibited (67, 247, 249). Ultimately, the presence of numerous mechanisms mediating exercise hyperemia emphasizes the importance of maintaining O_2 delivery to high-demand locomotive tissues during exercise (77, 248, 253).

Methodological considerations

There are methodological considerations that warrant discussion. First, we used propranolol HCl to non-selectively block all β-ARs in our participants as there is no selective β2-AR antagonist approved for human use. Thus, participants experienced significant decreases in heart rate and peak cardiac output due to the blockade of β1-ARs of the heart. However, we mitigated the impact of a reduced cardiac output by performing exercise in an isolated muscle group. In addition, acute treatment with intravenous propranolol HCL has previously been shown to not affect resting blood pressures (8). Indeed, the present study did not find any differences in resting or exercise blood pressure responses between CON and PROP, despite significant drops in heart rate. Second, there is a large variability in reported values of absolute $\dot{Q}_{LEG}$ during knee extension exercise. The present study found $\dot{Q}_{LEG}$ values slightly lower than Rådegran originally reported during exercise at ~20W (~ 2.7 vs. ~3.1 L/min) (91). However, many other studies consistently report lower absolute values at a similar exercise intensity (range: 1.1-1.8 L/min) (10, 98, 99). Ultimately, this might be reflective of different analysis strategies or exercise protocols. Nonetheless, our study employed a repeated measures design which would minimize such differences. Third, CON did not have a volume matched placebo control condition and participants

were not blinded to the propranolol condition. HCl propranolol was infused at a concentration of 2 mg/mL, so participants received on average 18.5 ± 3.25 mL and at most 25 mL of increase blood volume during PROP; considering an adult blood volume ranges from ~ 4-7 L, it is a negligible increase in blood volume. Fourth, we did not control for differences in menstrual phases in our female participants, where circulating estrogen would likely vary. To minimize the effects of varying estrogen levels, female participants in our study only performed experimental visits either during the early follicular or mid-luteal phase, so estrogen levels were kept consistent within each individual female participant. Furthermore, fluctuations in estrogen levels between menstrual phases do not appear to have a significant impact on exercise cardiovascular responses in young females (269). Fifth, quadriceps activity was significantly greater during PROP, however, authors are wary to speculate if hyperemia responses were impacted secondary to propranolol acting centrally on motor control as this was not the primary purpose of the study. Nonetheless, participants reaching similar peak workloads (Table 5) and no difference in oxygen demand (Figure 4) between CON and PROP visits suggest similar levels of muscle activity between visits.

3.5 Conclusion

Our study is the first to investigate the role of β_2-ARs in mediating possible sex differences in the exercise hyperemia response to incremental single leg knee extension exercise. Contrary to our prediction, we found no sex differences in the overall exercise hyperemia response and that β_2-AR play no significant role during the exercise hyperemia response in males and females. While β_2-ARs do not appear to significantly alter overall exercise hyperemia in either males or females, they play a distinct role in vascular regulation between the sexes. Specifically, β_2-AR blockade with propranolol had a more pronounced impact on FA dilation in females compared to males during

absolute exercise intensities, suggesting that β_2-ARs in females are crucial for regulating vascular tone in large conduit arteries during exercise. These findings contribute to the growing body of evidence suggesting that while β_2-ARs play a role in exercise-induced vasodilation particularly in females, their influence on overall hyperemia is limited. Our results align with the broader understanding that exercise hyperemia is mediated by numerous compensatory mechanisms to ensure sufficient supply of blood flow and O_2 delivery to active skeletal muscle.

Chapter 4: β-adrenergic receptors do not impact exercise hyperemia during rhythmic handgrip exercise in males and females

Doherty CJ, King TJ, Thompson BP, Chang J-C, Mann LM, Aguero AJ, Wojcik K, Carson I, Au JS, Foster, GE, Dominelli PB. β-adrenergic receptors do not impact exercise hyperemia during rhythmic handgrip exercise in males and females. Revisions requested *J Appl Physiol* ID#: JAPPL-00497-2026

4.1 Introduction

Exercise hyperemia is essential to ensure oxygen is sufficiently matched and delivered to active skeletal muscles (1). Indeed, blood flow increases linearly with progressive workloads in order to meet the skeletal muscle metabolic demand (92, 270, 271). Despite exercise hyperemia's importance, whether sex differences between males and females remains equivocal. Blood flow ($\dot{Q}_{ARM}$) and vascular conductance (VC_{ARM}) responses during dynamic exercise suggest a sex difference in the hyperemia response to exercise (10, 11, 272), whereas others demonstrated no differences (252, 260, 271, 273), even after adjusting for muscle mass (271).

Differences in beta-adrenergic receptor (β-AR) activity in the peripheral vasculature of females have been proposed as a potential mechanism responsible for sex differences in exercise hyperemia (10). Young females have a reduced sensitivity to norepinephrine-mediated vasoconstriction that was abolished with propranolol, a non-selective β-AR antagonist (8, 9). However, these findings identifying sex differences in β-AR activity on vascular function were performed at rest (8, 9). In contrast, few studies directly interrogate the role of β-AR in mediating the exercise hyperemia response. We recently reported no sex differences in the exercise hyperemia response to incremental knee extension exercise, irrespective of β-AR blockade with propranolol (271), while others found no differences in the exercise hyperemia response during rhythmic handgrip between males and females with β-AR blockade (273). However, a

retrospective study pooling results from three different studies found females to have an enhanced vasodilatory response attributable to β-AR activity during hypoxic low-intensity handgrip exercise (272). Interestingly, the contribution of β-ARs to the hyperemia response was diminished with increases in exercise intensity increased as the change in forearm vascular conductance response was not different with β-AR blockade (233, 254). Thus, β-AR-mediated sex differences in the exercise hyperemia response may only be detectable during small muscle mass, low-intensity hypoxic exercise.

The purpose of this study was to investigate the role of β-AR on exercise hyperemia in males and females during low-intensity rhythmic handgrip exercise to failure. We hypothesized that during low-intensity rhythmic handgrip exercise, the change in $\dot{Q}_{ARM}$ and VC_{ARM} would be blunted during β-AR blockade in females but not males. Despite potential sex differences in the role of β-AR in mediating exercise hyperemia, we also hypothesized that the overall hyperemia response, as determined by the linear slope of $\dot{Q}_{ARM}$ and VC_{ARM} responses over progressive exercise intensities, would not differ between males and females with or without β-AR blockade.

4.2 Methods

Sample size

An *a priori* sample size calculation was performed using data from a previous study that reported ȮARM responses at rest and during 10% MVC rhythmic handgrip exercise in males and females (272). Based on an effect size of $f = 0.78$, an α of 0.05, and a power of 0.80, a total sample size of 12 participants (6 per group) was required.

Table 6. Chapter 4 participant characteristics and resting baseline variables

Participant characteristics	Male (n=7)	Female (n=10)	P values	
Age (yr)	28 ± 6	27 ± 6	0.66	
Height (m)	1.80 ± 0.07	1.65 ± 0.03	< 0.01	
Body mass (kg)	76.4 ± 11.0	63 ± 3.6	< 0.01	
BMI ($kg \cdot m^{-2}$)	23.5 ± 2.4	23.2 ± 1.8	0.83	
Handgrip MVC (N)	495 ± 97	364 ± 70	< 0.01	
Baseline measures	Control		Propranolol	
	Males	Females	Males	Females
Heart rate (bpm) †	74 ± 8	70 ± 12	57 ± 5	53 ± 8
MAP (mmHg)	87 ± 9	79 ± 6	83 ± 13	79 ± 10
BA_D (mm) * †	4.2 ± 0.5	3.4 ± 0.2	4.3 ± 0.6	3.4 ± 0.3
$\dot{Q}_{ARM}$ ($ml \cdot min^{-1}$) *	46 ± 24	24 ± 17	47 ± 30	19 ± 9
VC_{ARM} ($ml \cdot min^{-1} \cdot mmHg^{-1}$) *	0.5 ± 0.3	0.3 ± 0.4	0.6 ± 0.5	0.3 ± 0.1
Shear rate (s^{-1})	11 ± 4	11 ± 9	10 ± 7	8 ± 3

Values are means ± SD. BMI, body mass index; MVC, maximal voluntary contraction; MAP, mean arterial pressure; BA_D, brachial artery diameter; $\dot{Q}_{ARM}$, arm blood flow; VC_{ARM}, arm vascular conductance. †, condition effect. *, sex effect. No significant sex × condition interaction was observed.

Participants

Seventeen healthy young adults (10 females) were recruited. Table 6 outlines participant characteristics and baseline variables. All participants were informed of experimental procedures and provided written informed consent. Participants were excluded if they had cardiovascular, respiratory, metabolic, or any other disease that would impact their response to exercise. Participants were nonsmokers and not taking any medication that would interact with propranolol. Finally, participants were restricted from exercise, recreational drugs, and alcohol consumption 24 h prior to testing visits. Female participants were regularly menstruating (n = 7) or using hormonal intrauterine device (n = 3). Both experimental testing visits for all females were performed during a similar menstrual phase 48-120 h apart (i.e., low-hormone early follicular phase or high-hormone mid-luteal phase). Of the regularly menstruating females, four were tested during the early follicular phase and three were tested during the mid-luteal phase. The study was approved the

University of Calgary Conjoint Health Research Ethics Board (REB24-1817) and University of British Columbia Clinical Research Ethics Board (H24-03148), both of which adhered to the Declaration of Helsinki, except for registration in a database.

Experimental overview

Each participant completed three testing visits. During the first testing visit, anthropometric characteristics were recorded, maximal voluntary contraction (MVC) efforts were measured using a digital hand dynamometer (MLT004/ST; ADInstruments, Sydney, NSW, Australia), and participants were familiarized with subsequent testing protocols. For the MVC, participants completed a minimum of 3 maximal handgrip efforts with their right hand and MVC was determined as the average of a 1-second peak across the two greatest efforts. The second and third testing visits were separated by 24-190 hours and were identical in protocol and differed only by the administration of HCl propranolol to cause β-AR blockade (PROP) or without HCl propranolol (control; CON). These visits consisted of an independent rhythmic handgrip exercise bout at a relative intensity of 10% of MVC followed by a progressive rhythmic handgrip exercise targeting workloads of 40N, 80N, 120N and finally 60% MVC until failure. The order of PROP and CON visits were randomized and stratified by sex.

Rhythmic handgrip exercise protocol

During the experimental visits 2 and 3, participants performed two rhythmic handgrip exercise protocols while continuously imaging of the brachial artery (BA) in the active arm and recording beat-by-beat cardiovascular variables (Figure 9). Following instrumentation, participants completed a 5-minute baseline measure in a seated upright position. During baseline and exercise, the participant's right arm was positioned perpendicular to the torso along the coronal

plane with a slight supination of the hand to permit ultrasound imaging, as depicted in Figure 9A. Rhythmic handgrip exercise was performed on the digital dynamometer (MLT004/ST) at a cadence of 30 contractions per minute at a duty cycle of 1:1 for every contraction and relaxation phase. The force output was displayed in real-time on a screen so participants could maintain the target grip force.

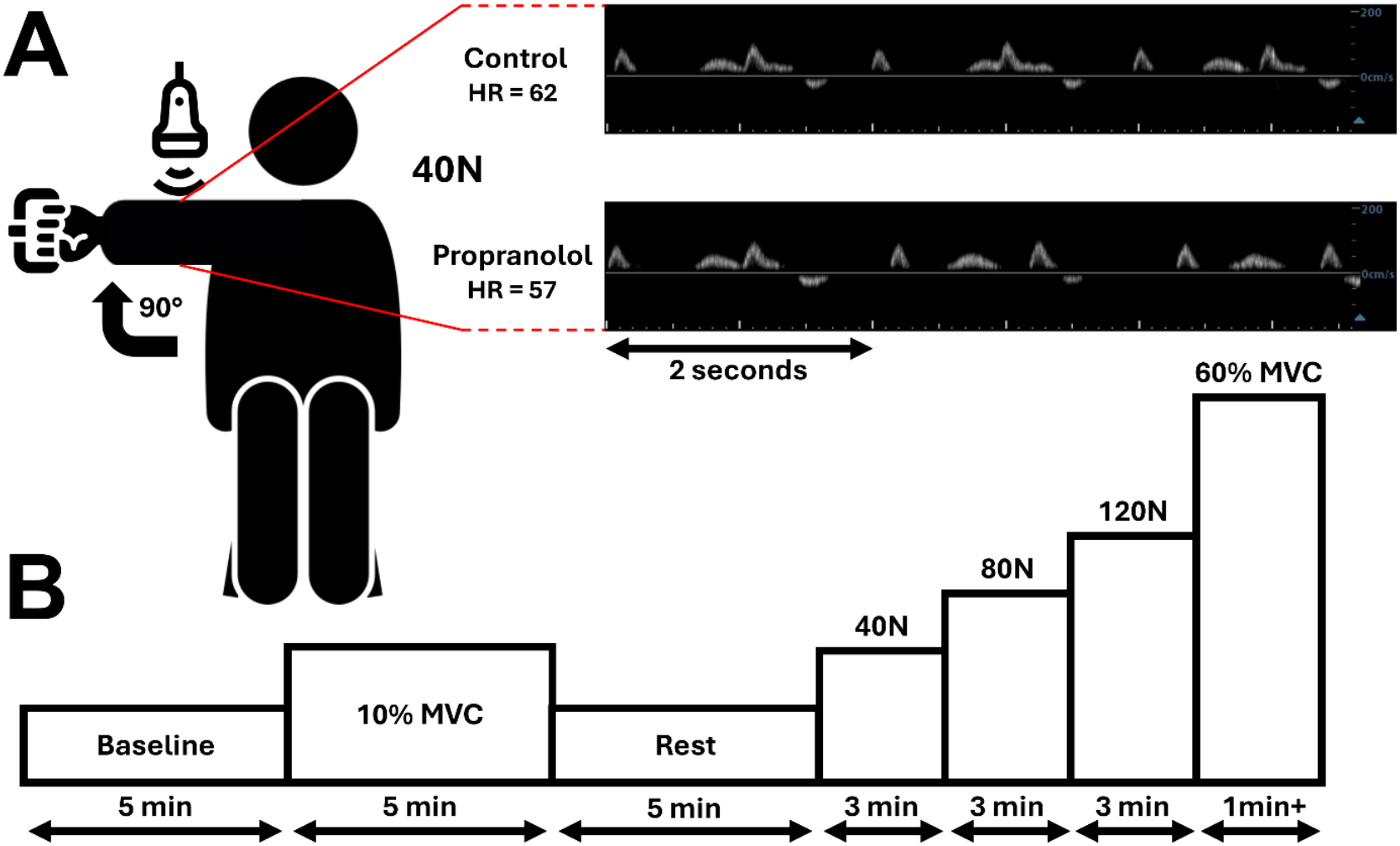


Figure 9. Representative brachial artery blood velocity tracing and rhythmic handgrip protocol

A representative brachial artery blood velocity profile during 40 N rhythmic handgrip exercise for control and propranolol visit (**A**) and the rhythmic handgrip exercise protocol performed on experimental visits (**B**).

The first handgrip protocol consisted of 5 minutes of rhythmic handgrip at 10% MVC. The 10% MVC condition was performed to investigate the role of β-AR in mediating the exercise hyperemia response during low-intensity non-fatiguing exercise. Following a 5-minute rest period, participants completed the second exercise protocol where they performed three, 3-minute stages of incremental rhythmic handgrip exercise protocol targeting 40, 80, and 120 N, followed by 60% MVC to volitional failure. All participants completed stages up to 120 N. However, 2 males and 3 females were unable to complete at least 1 minute of the 60% MVC condition. Instead, the peak handgrip intensity attained by each participant was pooled and analyzed as the absolute and relative change in cardiovascular variables from baseline (Table 7).

Propranolol administration

Beta-adrenergic receptor blockade was achieved by having participants take two oral doses of 80 mg propranolol HCl (160 mg total) prior to their PROP visit. The first dose was taken 7.5 hours before the visit, and the second dose was taken 1.5 hours prior to the start of the experimental testing visit to ensure peak blood propranolol concentrations occurred during the testing visit. A two-dose regimen was selected to ensure the first-pass metabolism of the liver was mitigated before taking the second dose (274). A single 80 mg oral dose of propranolol is effective in causing complete β-AR blockade, despite blood propranolol concentrations reaching only ~60-70 ng/ml (275). An equipotent propranolol metabolite 4-hydroxypropranolol is produced when taking oral propranolol, which is why complete blockade can be achieved at lower blood propranolol concentrations than the assumed 100 ng/ml blood propranolol concentration that is required during intravenous infusion of propranolol (221). Regardless, male and female participants in the present study were found to have no difference in the reduction in resting heart rate with propranolol (-Δ22 ± 6% vs. -Δ23 ± 6%, $p = 0.56$; males and females, respectively), which is congruent with

similar reductions in resting heart rate previously reported during intravenous beta-adrenergic blockade (range: ~-Δ15-25%) (8, 275, 276). Thus, given the two 80 mg doses along with the effect of the metabolites, we are confident that the propranolol dosing regimen was sufficient to cause complete blockade across our participants.

Measurements

Continuous measures of beat-to-beat blood pressure were collected by finger photoplethysmography on the participant's left hand (Human NINP Nano Interface; ADInstruments, Colorado Springs, CO), while heart rate was determined via electrocardiogram (ePM 12M, Mindray Bio-medical Electronics, Shenzhen, PR China). Absolute discrete measures of blood pressure at rest were taken from an automated oscillometric sphygmomanometer placed on the upper right arm of the participant (ePM 12M). Blood flow measurements in the right BA were made by duplex-mode ultrasound (M9 Ultrasound System, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, PR China) operating at 4.6-9MHz for B-mode and 3.6 MHz for Doppler mode from a site on the medial aspect of the right upper arm. Continuous measurements of both brachial artery diameter (BA_D) and blood velocity profiles were captured, with the latter collected at an insonation angle of <60°. Images of the artery were stored as video files and later analyzed offline using RadiAnt DICOM Viewer software (Medixant, Poznan, Poland). During the final minute of each stage, artery diameters were determined from the intima-lumen interface at both the distal and proximal vessel walls at end-diastole during the relaxation phase of the duty cycle. Three measures of BA_D were made across six frames (18 total measurements) and averaged for each stage. Doppler flow velocity profiles were analyzed offline using an open-source MATLAB program (FloWave; Vanderbilt University Institute of Imaging Science, Nashville,

TN), which quantifies pixel intensity variations over time, providing a measure of the intensity-weighted mean blood flow velocity (277).

Data analysis

All cardiovascular and arm blood flow were calculated as 30 s averages during the final minute of baseline and each exercise stage. All continuous signals were collected at 200 Hz and recorded using PowerLab (ADInstruments, Sydney, NSW, Australia). Arm blood flow ($ml \cdot min^{-1}$) was calculated by the following equation:

$$\dot{Q}_{ARM} = MBVel\ (cm \cdot s^{-1}) \times \pi \times BAradius^2(cm^2)\ \times 60\ (s \cdot min^{-1}) \quad (6)$$

Where MBVel is mean blood velocity ($cm \cdot s^{-1}$), and BA radius was derived from BA_D. Arm vascular conductance was calculated as the quotient of $\dot{Q}_{ARM}$ and mean arterial pressure (MAP). Shear rate was calculated by: (8 × MBV)/BA. To account for differences in absolute strength, measures of cardiovascular and local tissue perfusion were also quantified relative to each participant's MVC. Measured outcomes were also examined as both absolute and relative changes from baseline.

Statistical analysis

All statistical analyses were performed using GraphPad Prism Software (GraphPad Prism 10.4.1, La Jolla, CA). Sex differences in participant characteristics were assessed using unpaired Student's *t* tests. Baseline variables were assessed using a 2-way ANOVA (sex × condition). Likewise, absolute and relative changes in variables from baseline to 10% MVC and from baseline to individual peak exercise intensity achieved were assessed separately using a 2-way ANOVA (sex × condition). Absolute variables at baseline, and during 40, 80 and 120N were assessed using a 3-way ANOVA analysis (sex × condition × exercise intensity). Similarly, the relative change in

variables from baseline was assessed using a 3-way ANOVA analysis (sex × condition × exercise intensity). No significant 3-way interaction between factors was observed significant across each variable (all, $p > 0.24$), thus these findings were not reported in the results. *Post hoc* assessment of interactions was completed using a Tukey's HSD test. A summary of the main effects of sex, condition, and exercise intensity and the sex × condition interaction may be found at the top of each figure.

Exercise intensity was eliminated as a factor by examining the slope response between the change in $\dot{Q}_{ARM}$ and VC_{ARM} and absolute target handgrip force. Slope responses for the relative change in $\dot{Q}_{ARM}$ and VC_{ARM} responses over target handgrip force relative to MVC were also examined to account for differences in strength and individual baseline differences. Examining exercise hyperemia slope responses permitted for a closer examination of the relationship between sex and β-AR blockade with propranolol. Slopes were derived from the 10% MVC, 40 N, 80 N and 120 N exercise stages. Slope responses were assessed using at 2-way ANOVA (sex × condition) with post hoc Bonferroni Student's *t* test. Plotting of modelled individual and mean linear regression slopes of absolute and relative changes in $\dot{Q}_{ARM}$, VC_{ARM} over absolute increases in force and increases in force relative to handgrip MVC were generated using R (version 4.4.2) and RStudio. The acceptable type I error rate was set at $\alpha=0.05$ for all analyses.

4.3 Results

Participant characteristics and baseline measurements

Participant characteristics are presented in Table 6. Age and body mass index did not differ between males and females (both $p > 0.05$). Males were significantly taller ($p < 0.01$) and heavier

($p < 0.01$) than females. Handgrip MVC was significantly greater in males compared with females ($p < 0.01$). Baseline hemodynamic measures can be found in Table 6. A main effect of condition was observed with heart rate, being lower during PROP for both males and females ($p < 0.01$). In contrast, MAP and shear rate did not differ between condition or sex (both $p > 0.05$). A significant effect of sex was observed for BA_D, $\dot{Q}_{ARM}$, VC_{ARM}, with males exhibiting larger baseline values versus females (all $p < 0.05$). A main effect of condition for BA_D resulted in greater diameters during the PROP experimental visit ($p < 0.05$). No significant interaction between sex and propranolol was observed for BA_D at baseline ($p > 0.05$).

10% MVC exercise protocol

Absolute and relative changes in cardiovascular variables from baseline to the 10% MVC are presented in Table 7. The absolute increase in heart rate and MAP from baseline to the low-intensity exercise condition was not different by sex or condition (all, $p > 0.05$). A significant condition effect was observed for the absolute change in BA_D, with greater dilation observed with propranolol ($p = 0.02$). The absolute change in shear rate during exercise was not different by sex or condition ($p > 0.05$). The relative change in heart rate, MAP and shear rate did not differ by sex or condition (all, $p > 0.05$). The relative change in BA_D mirrored the absolute change in BA_D as increased dilation was found during PROP ($p = 0.04$).

Table 7. Absolute and relative change in cardiovascular responses from baseline to 10% MVC.

Absolute changes	Control		Propranolol	
	Males	Females	Males	Females
Δ Heart rate (bpm)	2 ± 6	2 ± 4	0 ± 2	1 ± 4
Δ MAP (mmHg)	6 ± 4	6 ± 4	8 ± 4	7 ± 6
Δ BA_D (mm) †	0.03 ± 0.04	0.02 ± 0.07	0.09 ± 0.06	0.03 ± 0.06
Δ Shear rate (s^{-1})	14 ± 5	17 ± 5	11 ± 4	16 ± 9
Relative changes	Control		Propranolol	
	Males	Females	Males	Females
Δ Heart rate (%)	3 ± 8	3 ± 6	-1 ± 3	2 ± 7
Δ MAP (%)	8 ± 5	7 ± 5	10 ± 5	11 ± 9
Δ BA_D (%)†	1 ± 1	0 ± 2	2 ± 2	1 ± 2
Δ Shear rate (%)	149 ± 88	214 ± 101	158 ± 114	249 ± 238

Values are means ± SD. MVC, maximal voluntary contraction; MAP, mean arterial pressure; BA_D, brachial artery diameter; †, condition effect. No significant sex × condition interaction was observed.

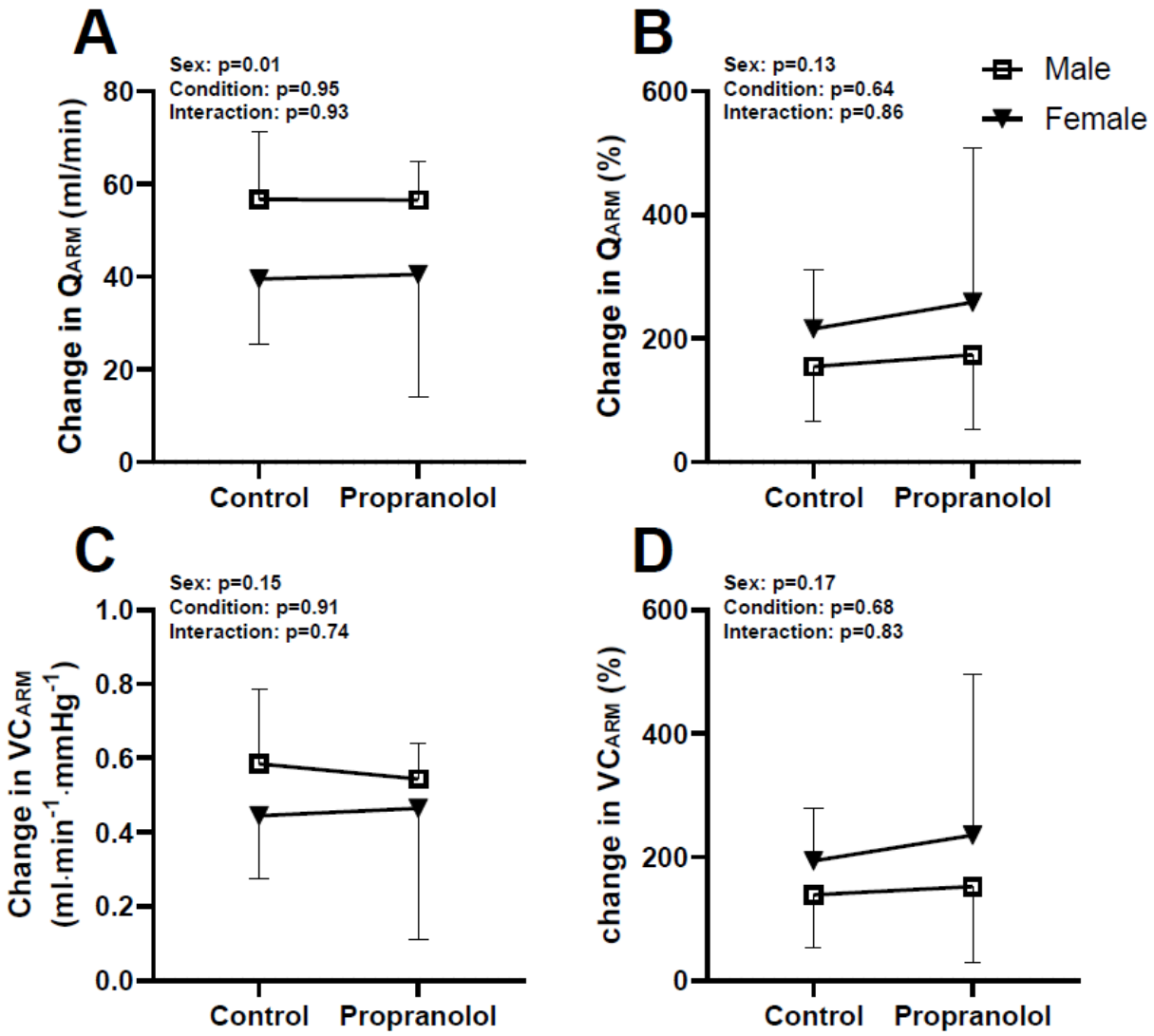


Figure 10. Change in blood flow and vascular conductance response during 10% MVC rhythmic handgrip exercise.

Absolute (**A**) and relative (**B**) change in blood flow to the arm from baseline to 10% MVC rhythmic handgrip exercise. Absolute (**C**) and relative (**D**) change in vascular conductance to the arm from baseline to 10% MVC rhythmic handgrip exercise. Males and females denoted by white squares and black triangles, respectively.

The absolute and relative changes in $\dot{Q}_{ARM}$ and VC_{ARM} from baseline to the 10% MVC rhythmic handgrip condition are displayed in Figure 10. Males had a greater absolute increase in $\dot{Q}_{ARM}$ compared to females, but this was not different by condition. When the change in $\dot{Q}_{ARM}$ was examined relative to baseline, the sex effect was abolished, and no condition effect persisted (both, $p > 0.05$). Both absolute and relative changes in VC_{ARM} were not different by sex or condition effect (both, $p > 0.05$).

Incremental exercise protocol

Figure 11 displays the absolute change in hemodynamics at baseline and during rhythmic handgrip exercise targeting 40, 80, 120 N. Across all variables, exercise intensity elicited increases in heart rate, MAP, BA_D, $\dot{Q}_{ARM}$, shear rate, and VC_{ARM} (all, $p < 0.01$). A main effect of sex was observed for several variables: males exhibited larger BA_D, higher $\dot{Q}_{ARM}$, reduced shear rates, and higher VC_{ARM} (all, $p < 0.05$). Propranolol significantly reduced heart rate, $\dot{Q}_{ARM}$, and shear rate ($p < 0.05$). A significant sex × condition interaction was observed for BA_D ($p = 0.02$), where propranolol increased absolute BA_D in males ($p=0.02$), but not in females ($p=0.99$) throughout exercise.

Figure 12 shows the relative change in hemodynamics at baseline and during rhythmic handgrip exercise targeting 40, 80, 120 N. Again, a main effect of exercise intensity observed across all variables relative to baseline (all, $p < 0.01$). Females were found to have larger relative increases in heart rate, $\dot{Q}_{ARM}$, shear rate and VC_{ARM} (all, $p < 0.05$). Propranolol did not affect the percent change from baseline in any variable, except for BA_D, which showed a significant sex × condition interaction ($p=0.03$) where propranolol blunted the increase in BA_D in females ($p = 0.03$), but not males ($p = 0.96$).

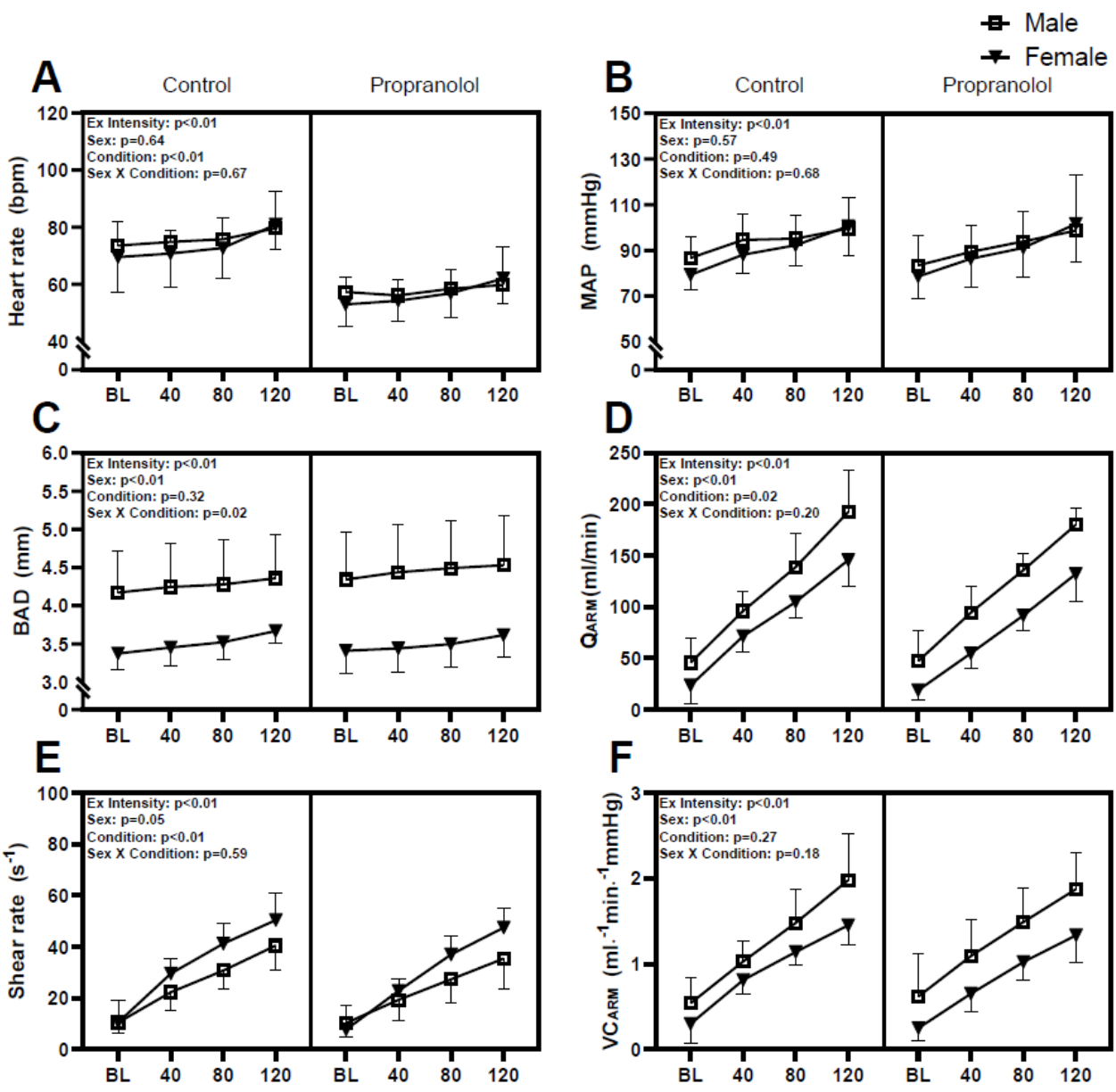


Figure 11. Absolute cardiovascular and arm perfusion responses to incremental rhythmic handgrip exercise.

Absolute heart rate (**A**), mean arterial pressure (MAP) (**B**), brachial artery diameter (BA_D) (**C**), arm blood flow ($\dot{Q}_{ARM}$) (**D**), shear rate (**E**), and arm vascular conductance (VC_{ARM}) (**F**) responses at baseline and during 40, 80, and 120 N rhythmic handgrip exercise. Males and females denoted by white squares and black triangles, respectively.

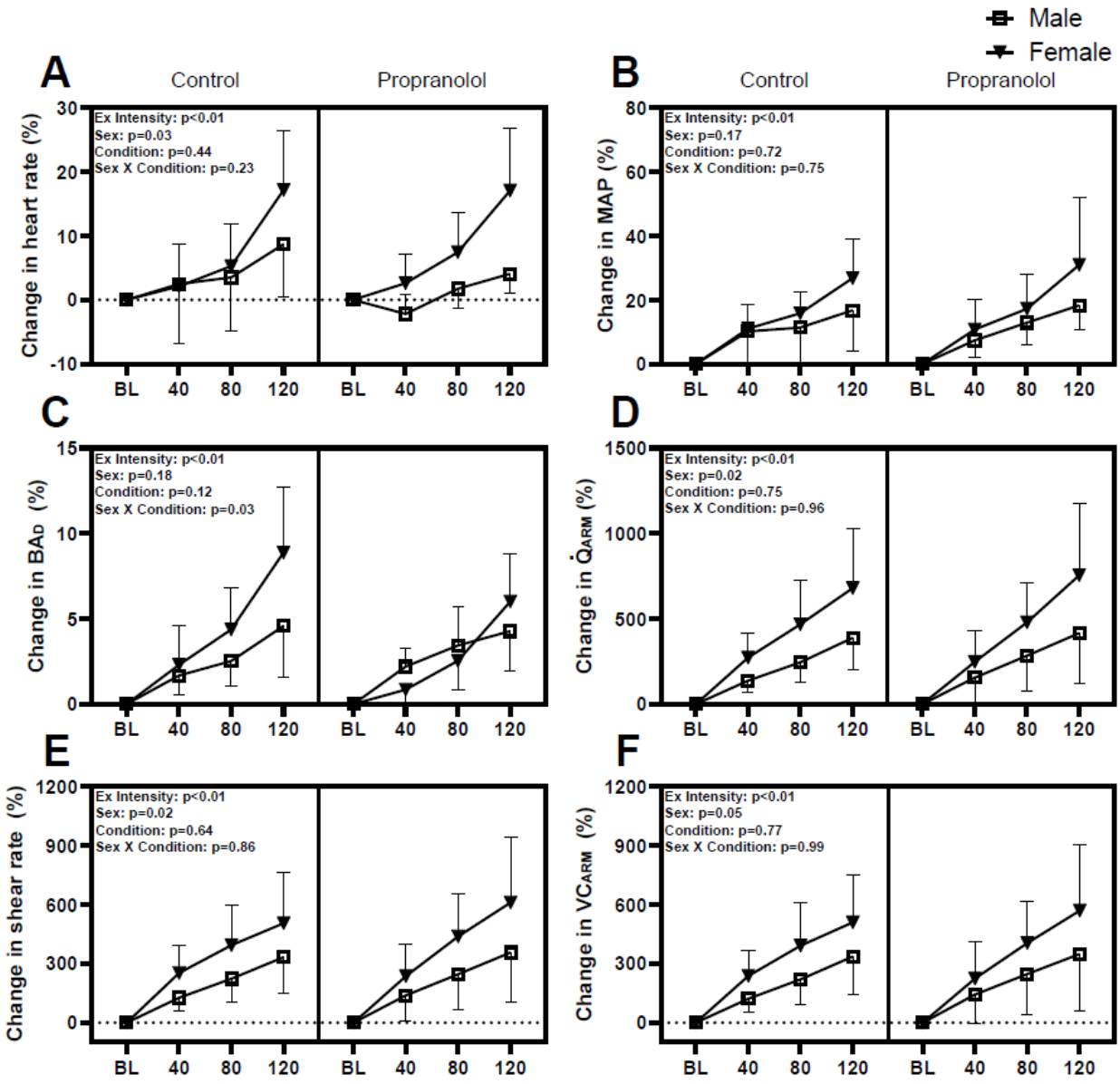


Figure 12. Cardiovascular and arm perfusion responses relative to baseline to incremental rhythmic handgrip exercise.

Change in heart rate (**A**), mean arterial pressure (MAP) (**B**), brachial artery diameter (BA_D) (**C**), arm blood flow ($\dot{Q}_{ARM}$) (**D**), shear rate (**E**), and arm vascular conductance (VC_{ARM}) (**F**) responses relative to baseline during 40, 80, and 120 N rhythmic handgrip exercise. Males and females denoted by white squares and black triangles, respectively.

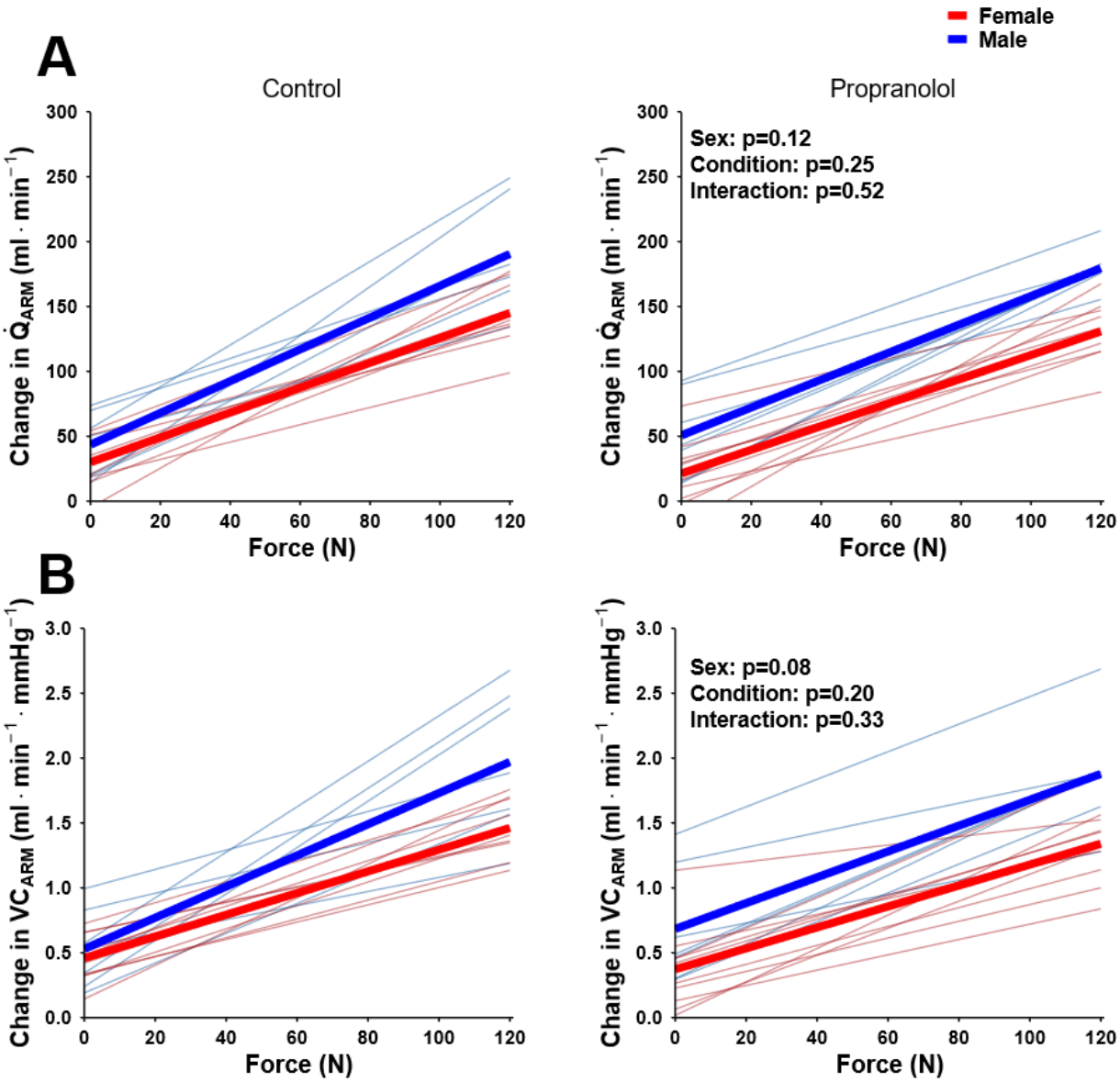


Figure 13. Mean linear regression model of blood flow and vascular conductance response over absolute handgrip force.

Mean linear regression of absolute changes in arm blood flow ($\dot{Q}_{ARM}$) (**A**) and arm vascular conductance (**B**) over absolute handgrip force in males (blue) and females (red) during control and propranolol conditions. Bolded lines represent the mean linear regression curves.

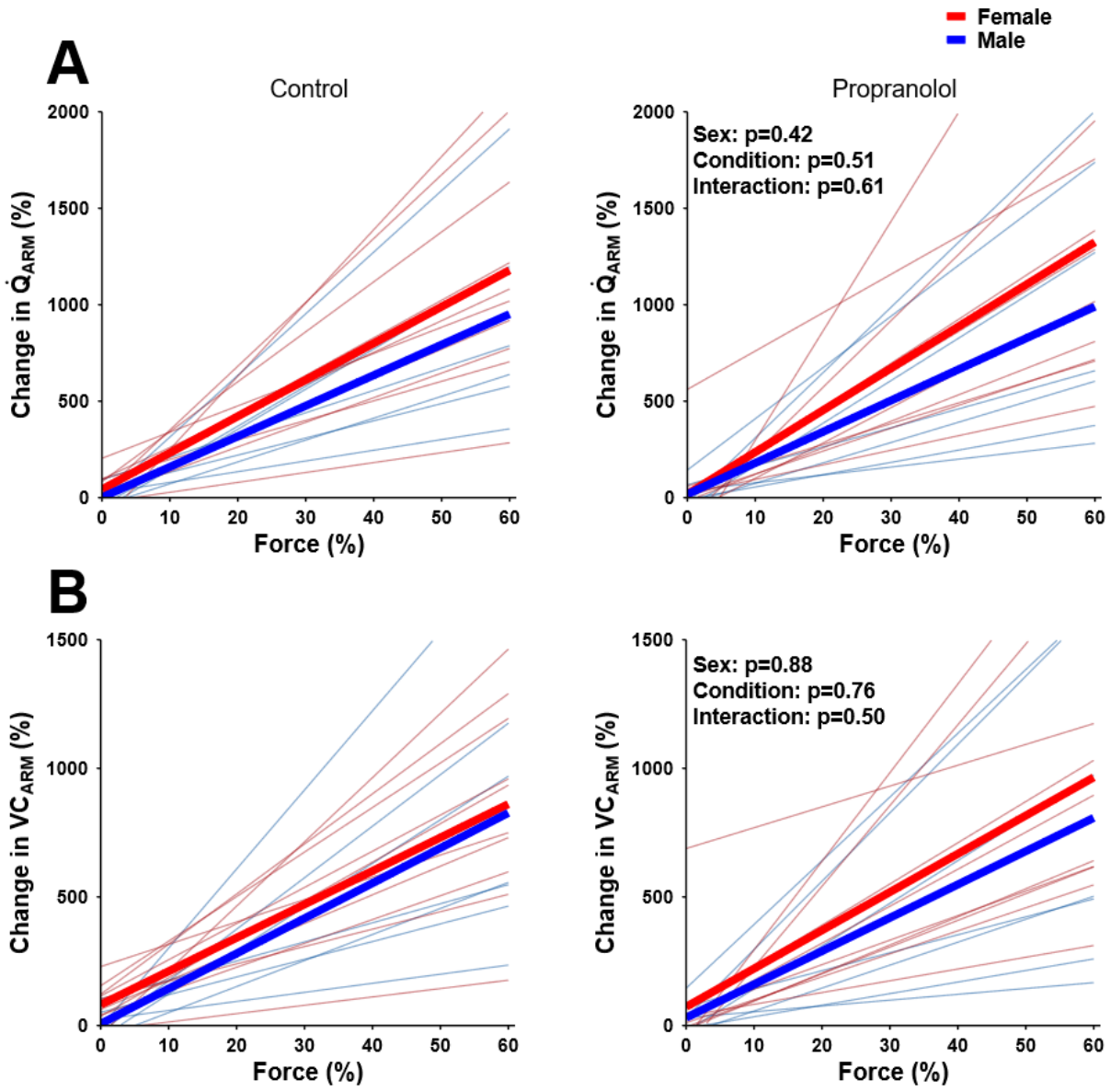


Figure 14. Mean linear regression model of the relative change in blood flow and vascular conductance to the arm over handgrip force relative to maximal handgrip effort

Mean linear regression of the change in arm blood flow ($\dot{Q}_{ARM}$) (A) and arm vascular conductance (B) relative to baseline over handgrip force relative to maximal handgrip effort in males (blue) and females (red) during control and propranolol conditions. Bolded lines represent the mean linear regression curves.

Figure 13 outlines modelled individual and mean linear regression slopes of absolute changes in $\dot{Q}_{ARM}$, VC_{ARM} over absolute increases in force in males and females. Absolute $\dot{Q}_{ARM}$ and VC_{ARM} slope responses were not different by sex or condition (all $p > 0.05$). Figure 14 displays the modelled individual and mean linear regression slopes of the percent change of $\dot{Q}_{ARM}$ and VC_{ARM} over increases in target force relative to handgrip MVC. Unsurprisingly, after adjusting for both strength and individual baseline differences, slope responses did not differ between sexes or conditions (all, $p > 0.05$).

Peak exercise

Absolute changes in central hemodynamics and the exercise hyperemia response from baseline to peak exercise are presented in Table 8. The absolute increase in heart rate from baseline to peak exercise was blunted with propranolol in both males and females ($p < 0.05$). Conversely, the absolute increase in MAP also exhibited a significant condition effect, with greater increases observed during PROP compared to control ($p < 0.05$). Changes in absolute BA_D and shear rate from baseline to peak exercise did not differ by sex or condition ($p > 0.05$). A main effect of sex was observed for absolute changes in $\dot{Q}_{ARM,}$ with males demonstrating greater increases than females across conditions ($p < 0.05$). However, the absolute change in VC_{ARM} was not different by sex or condition ($p > 0.05$).

Relative changes in hemodynamics from baseline to peak exercise are also found in Table 8. The relative increases in heart rate and MAP did not differ by sex or condition (all, $p > 0.05$). Similarly, the relative change in BA_D and shear rate was not different by sex or between conditions (all $p > 0.05$). Changes in $\dot{Q}_{ARM}$ and VC_{ARM} relative to baseline were not different between sexes and or between conditions.

Table 8. Absolute and relative change in cardiovascular responses from baseline to peak exercise during rhythmic handgrip exercise

Absolute changes	Control		Propranolol	
	Males	Females	Males	Females
Δ Heart rate (bpm) †	19 ± 12	26 ± 14	14 ± 7	18 ± 12
Δ MAP (mmHg) †	31 ± 11	32 ± 7	39 ± 13	35 ± 11
Δ BA_D (mm)	0.29 ± 0.18	0.40 ± 0.12	0.30 ± 0.18	0.28 ± 0.09
Δ $\dot{Q}_{ARM}$ ($ml \cdot min^{-1}$) *	229 ± 71	168 ± 52	223 ± 72	142 ± 41
Δ VC_{ARM} ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	1.84 ± 0.74	1.43 ± 0.52	1.63 ± 0.53	1.21 ± 0.39
Δ Shear rate (s^{-1})	43 ± 18	49 ± 11	36 ± 14	46 ± 10
Relative changes	Control		Propranolol	
	Males	Females	Males	Females
Δ Heart rate (%)	28 ± 18	38 ± 21	26 ± 14	35 ± 22
Δ MAP (%)	38 ± 15	40 ± 9	46 ± 14	46 ± 15
Δ BA_D (%)	7 ± 5	12 ± 3	7 ± 4	8 ± 3
Δ $\dot{Q}_{ARM}$ (%)	625 ± 388	901 ± 393	693 ± 525	884 ± 388
Δ VC_{ARM} (%)	440 ± 305	624 ± 302	452 ± 392	591 ± 283
Δ Shear rate (%)	483 ± 298	616 ± 278	503 ± 353	676 ± 319

Values are means ± SD. MAP, mean arterial pressure; BA_D, brachial artery diameter; $\dot{Q}_{ARM}$, arm blood flow; VC_{ARM}, arm vascular conductance. †, condition effect. *, sex effect. No significant sex × condition interaction was observed.

4.4 Discussion

The findings of this investigation were fourfold. First, absolute $\dot{Q}_{ARM}$ during exercise were greater in males than in females for a given absolute workload. However, these differences were abolished when adjusted for baseline values and handgrip MVC, suggesting sex-related differences in absolute $\dot{Q}_{ARM}$ and VC_{ARM} are largely attributable to size and strength differences. Second, contrary to our hypothesis, β-AR blockade did not alter $\dot{Q}_{ARM}$ or VC_{ARM} responses between males and females during low-intensity (10% MVC) rhythmic handgrip exercise. Third, consistent with our hypothesis, the slope of the relationship between the hyperemic response and exercise intensity was comparable between males and females regardless of β-AR blockade. Finally, we observed greater vasodilation of the BA in females compared to males, which was blunted to a greater extent

with propranolol. This provides further support for the involvement of local β2-adrenergic receptors (β_2-AR) in the dilation of conduit arteries during exercise (271). Current findings suggest the exercise hyperemia response is not different between males and females and that the magnitude of the hyperemic response during incremental exercise is largely independent of β-AR activity. Altogether, our study adds to the growing body of evidence highlighting that the fundamental relationship between metabolic demand and limb perfusion during exercise is preserved irrespective of sex.

Sex differences in exercise hyperemia

Whether sex differences exist in exercise hyperemia remains unresolved. Previous work found females to have greater blood flow and vascular conductance to active muscles during rhythmic handgrip (272), dynamic knee extension exercise (10, 11) and cycling exercise (278). In contrast, there is a growing number of studies suggesting the exercise hyperemia response is not different between males and females regardless of modality (252, 260, 271, 273, 279, 280). Presently, we found males to have greater absolute $\dot{Q}_{ARM}$ and VC_{ARM} across exercise intensities (Figure 11). However, these differences were eliminated when hyperemia responses were adjusted for individual resting baseline $\dot{Q}_{ARM}$ and VC_{ARM} measures (Figure 12). These findings support the notion that apparent sex differences in exercise hyperemia during dynamic exercise are primarily explained by allometric differences in body size, muscle mass, and strength, rather than fundamental differences in vascular control. In addition, when we examined male and female slope responses of $\dot{Q}_{ARM}$ and VC_{ARM} over progressive workloads, no differences were observed even after adjusting for strength and baseline differences between participants (Figures 5, 6). These results align with previous work from our group, which found hyperemia responses to the leg did

not differ between males and females when normalized to peak exercise intensity, relative workload or muscle mass (271).

β-adrenergic receptors and exercise hyperemia

Increased estrogen levels in females have previously been suggested to increase β_2-AR activity in the peripheral vasculature (265). An estrogen-related mechanism governing sex differences in the exercise hyperemia response was observed when older post-menopausal females had blunted limb blood flow and vascular conductance responses compared to young females after adjusting for individual peak exercise intensity and muscle mass (11). Other work performed at rest found young females to exhibit reduced α-adrenergic vasoconstrictor responsiveness and enhanced β-AR–mediated vasodilation compared with males (8, 9). The present study aligns with recent work refuting the notion that sex differences in hyperemia can be explained by differences in β-AR activty (271, 273). Indeed, β-AR blockade with propranolol did not meaningfully alter the overall exercise hyperemia response in males or females. This preservation of the hyperemia response in both sexes during PROP can be explained by the numerous hyperemia mechanisms, which remain active in the face of systemic β-AR blockade.

More recently, Maier and colleagues found $\dot{Q}_{ARM}$ and VC_{ARM} responses to rhythmic handgrip exercise at 25% MVC did not differ between sexes in the presence of β-AR blockade at sea level and at 3800 m altitude (273). Other work examining the role of β-AR in mediating exercise hyperemia in hypoxic conditions observed that females had a greater reliance on β-ARs to mediate the exercise hyperemia response, which was suggested during low-intensity rhythmic handgrip exercise (10% MVC) but not during higher intensity exercise (20% MVC) in hypoxic conditions (272). The present study did not observe any differences in blood flow or vascular conductance to the arm in males and females with β-AR blockade during the 10% MVC rhythmic

handgrip condition (Figure 10). Altogether, β-AR appears to have an enhanced role in mediating exercise hyperemia at low-exercise intensity in hypoxic conditions, but its role in normoxic conditions is limited. The increased reliance on β-AR receptors to mediate vasodilation in hypoxia may be explained by two different mechanisms. First, blood oxygen content is lower with hypoxia, and greater absolute blood flows are required to deliver the same amount of oxygen to exercising tissue for a given workload. Therefore, the contribution of beta-receptors during low-intensity exercise may simply be more easily detected due to the greater changes in blood flow in hypoxia. Second, hypoxia is known to cause significant increases in sympathetic activity at rest and during exercise (281). Thus, an increased sympathetic state could potentially result in a greater reliance on β_2-AR vasodilation to mediate the hyperemia response during low-intensity exercise. Regardless, future studies examining β-AR signaling under conditions of altered oxygen availability may provide further insight into the contexts in which β-AR mechanisms meaningfully influence skeletal muscle perfusion.

Brachial artery diameter

In examining relative changes in BA_D (Figure 12C), propranolol was found to blunt BA_D in females, but not males. This finding aligns with previous work, which found enhanced femoral artery dilation in females but not males during incremental knee extension exercise (271). Additionally, shear rate did not change with propranolol (Figure 12E), despite the blunted brachial artery response in females. Instead, the present study results add to the evidence of a sex difference in β-AR vasodilator responsiveness (8, 181, 264). Indeed, a rodent model found enhanced arteriole dilation in females but not males (264), and in humans, young females were found to have a reduced vasoconstrictor response to alpha-adrenergic mediated vasoconstriction during β-AR blockade (8). Importantly, these diameter changes did not translate into meaningful differences in

downstream perfusion. Neither $\dot{Q}_{ARM}$ nor VC_{ARM} responses were differentially affected by propranolol between sexes, suggesting local β_2-AR are not critical to mediating the overall hyperemia response during exercise. Altogether, these findings suggest females experience greater conduit artery dilation in response to exercise hyperemia, but these changes appear to have a minimal impact on the integrated hyperemia response to exercise.

Implications for exercise hyperemia

The present study did not observe a sex-based difference in the overall exercise hyperemia response to rhythmic handgrip exercise, suggesting that β_2-AR-mediated sex differences in vasodilatory responsiveness observed at rest (8, 9) are insignificant in the hyperemia response to exercise. Our findings also refuted previous work suggesting an enhanced role for β-AR in mediating the exercise hyperemia response to low-intensity small-muscle mass exercise (233). Instead, our results support the notion that numerous compensatory mechanisms are activated in both sexes during exercise to ensure adequate oxygen delivery to in-demand tissues. Indeed, other studies blocking a single known vasodilator pathway similarly found no change in the exercise hyperemia response (247, 249). Only when multiple vasodilator pathways were pharmacologically blocked was the exercise hyperemia response reduced (247, 248). Altogether, the presence of multiple mechanisms mediating exercise hyperemia highlights the importance of preserving oxygen delivery to locomotive tissues during exercise (248, 282).

Methodological considerations

There are methodological considerations that warrant mention. First, absolute arm blood flow values during the rhythmic handgrip exercise were lower than what is normally reported in the literature (92, 272). However, our rhythmic exercise was performed on a static digital

dynamometer, which senses changes in grip forces without any movement of the hand. Limited movement could reduce the mechanical muscle pump action and reduce blood flow to the active limb. In addition, our experiments were performed in the seated position with the hand positioned above the level of the heart. Most studies employing a rhythmic handgrip protocol are generally performed in the supine position, mitigating the effect of gravity on arm perfusion. Taken together, the increased influence of gravity and limited movement between contraction and relaxation phases could explain the blunted absolute blood flow responses. Regardless, the relationship between blood flow responses in both males and females remained linear across increasing exercise intensities. Second, a selective β_2-AR antagonist is not yet approved for use in humans, thus, HCl propranolol, a non-selective β-AR antagonist, was given to participants to examine the role of local β_2-AR receptors in mediating the exercise hyperemia response in peripheral blood vessels. Systemic β-AR blockade is known to blunt cardiac output and limit exercise capacity, particularly during progressive whole body exercise to fatigue (238). However, present study selected a single-arm rhythmic handgrip model to ensure the exercise hyperemia response would not be limited by the reduction of cardiac output during systemic blockade. In addition, the absolute change in heart rate to rhythmic handgrip exercise was not found to be different between CON and PROP visits, suggesting the rhythmic handgrip protocol was not cardiac output limited during β-AR blockade.

4.5 Conclusion

We examined the role of beta-adrenergic receptors to the exercise hyperemia response to the active arm during rhythmic handgrip exercise. We did not observe any sex differences in the exercise hyperemia response to low-intensity small muscle mass exercise or to the overall exercise

hyperemia response. Nor did we observe any differences in the exercise hyperemia response in males or females during β-AR blockade. However, β-AR appear to play a role in the vascular regulation between sexes as females were found to have a blunted BA_D response with propranolol relative to males, suggesting β-AR may be crucial in females to regulate vascular tone in large conduit arteries during exercise. Ultimately, our findings support the concept that redundant compensatory vasodilatory mechanism preserve perfusion of skeletal muscles, irrespective of sex or β-AR activity to ensure adequate blood supply and oxygen delivery to active skeletal muscle.

Chapter 5: Impact of β-adrenergic receptors on the diaphragm exercise hyperemia response in males and females

Doherty CJ, Thompson BP, Aguero AJ, Chang J-C, Mann LM, Wojcik K, Ghitter R, Isidori AL, Carson I, Lipp CT, Roy S, Lance ML, King TJ, Au JS, Foster GE, Dominelli PB. Impact of β-adrenergic receptors on diaphragm exercise hyperemia in males and females. In preparation for submission to *J Physiol.*

5.1 Introduction

The diaphragm is a vital skeletal muscle due to its central role in driving respiration. Unlike other skeletal muscles, the diaphragm is continuously active to ensure adequate alveolar ventilation and gas exchange in the lungs. The vasculature of the diaphragm is less sensitive to α1-adrenergic receptors (AR) mediated vasoconstriction (114) and the blunted sensitivity is thought to be a means to preserve blood flow to the diaphragm in the face of increased sympathetic activity (*e.g.*, exercise) (114). Nonetheless like all other skeletal muscles, the diaphragm requires commensurate increases in blood flow with increasing work to ensure sufficient delivery of oxygen to the exercising tissue (111, 283).

Prominent sex-differences in the respiratory system exist. Compared to height- and lung sized-matched males, females have smaller conducting airways resulting in a greater work of breathing (157, 199, 284) and increased oxygen consumption to the respiratory muscles during exercise (211). Baring changes in extraction, muscle oxygen consumption is proportional to its delivered fraction of cardiac output. As such, it could be hypothesized that females dedicate a greater fraction of cardiac output to their respiratory muscles. Sex differences also exist in the cardiovascular system of healthy young adults. Females generally have lower blood pressure at rest and a reduced sensitivity to alpha-adrenergic mediated vasoconstriction at rest (6, 8, 9, 251).

However, whether sex differences in the hyperemic response to exercising skeletal muscle remains equivocal (10, 11, 137, 183, 185, 252, 260, 271)

A likely mechanism responsible for the sex-differences in blood pressure regulation at rest is the upregulation of β_2-ARs in females due to the greater presence of estrogen. Hart et al. provided evidence for this by demonstrating that the administration of propranolol, a non-selective β-AR antagonist, in females increased vasoconstrictor sensitivity to levels observed in males and post-menopausal females (8). However, the limited studies to have directly investigated have not found a role for β-AR in exercise hyperemia to active locomotor muscles in either males or females (271, 273).

Little is known about the role of β_2-AR in mediating diaphragm perfusion. Given the unique vascular properties of the diaphragm during exercise and the sex differences of the respiratory system, it is unknown if β_2-ARs mediate the hyperemic response in the respiratory muscles during exercise. Thus, the purpose of the present study is to investigate the role of β_2-AR in young males and females on blood flow and vascular conductance to the diaphragm at rest and during graded inspiratory threshold loading. It was hypothesized that the $\dot{Q}_{DIA}$ and diaphragm vascular conductance (DVC) response would not be different between sexes and that this response would not be unchanged with β-AR blockade.

5.2 Methods

Sample size

When this study was designed, no previous work had used CEUS to directly examine sex differences in diaphragm blood flow or skeletal muscle blood flow in humans. Therefore, our

sample size calculation was based on a previous study that reported sex differences in the hyperemic response to dynamic knee extension exercise (10). Using the reported Cohen's *f* effect size of 0.45, an alpha of 0.05, and a power of 0.80, a sample size of seven participants per group (14 total participants) was recommended.

Participants

Fourteen healthy young adults (7 females) were recruited. Table 9 outlines participant characteristics. Female participants were regularly menstruating (n = 5) or using hormonal intrauterine device (n = 2). Both experimental testing visits for all females were performed during a similar menstrual phase 48-120 h apart (i.e., low-hormone early follicular phase or high-hormone mid-luteal phase). Of the regularly menstruating females, three were tested during the early follicular phase and two were tested during the mid-luteal phase. Participants were screened for cardiovascular, respiratory, metabolic or any other disease that would impact their response to exercise. Participants were nonsmokers and were not on any medication that would interact with HCl propranolol. Prior to testing visits, participants restricted from exercise, recreational drugs, and alcohol consumption. Study was approved by the University of Calgary Conjoint Health Research Ethics Board (REB24-1817), which adheres to the Declaration of Helsinki, except for registration in a database. All participants were informed of experimental procedures and provided written informed consent.

Experimental overview

Each participant completed three testing visits. During visit 1, participants were familiarized with the inspiratory threshold loading (ITL) protocol, performed forced vital capacity maneuvers, and completed a maximal exercise test on a cycle ergometer. During the experimental

testing visits 2 and 3, participants completed the experimental protocol on the ITL device while continuous cardiorespiratory variables were assessed following oral administration of HCl propranolol (PROP) or without (control, CON). The order between CON and PROP visits was randomized between participants, stratified by sex.

Familiarization, spirometry assessment and $\dot{V}O_{2peak}$ testing visit (Day 1)

Upon completion of the informed consent and health screening questionnaire, participant height and body mass were measured and body mass index was derived (kg/m^2). Afterwards, participants were familiarized with a custom ITL device outlined by others (285). Figure 15A provides an illustration of the cardiorespiratory measures, a schematic of an ITL device and a representative sample of a diaphragm pressure (P_{DIA}) guideline and tracing visualized by the participant. Participants inspired against a weighted valve, with increases in weight corresponding to increases in inspiratory effort. Participants then completed three trials on the device to determine the necessary weight required to achieve the desired P_{DIA} targets of 15, 30, and 45 cmH_2O on the ITL device. Breathing rate was fixed at 15 breaths/min and targeted a 1:1 duty cycle for inspiration and expiration.

Following familiarization with ITL device, participants performed forced vital capacity (FVC) maneuvers. Expired air flows were assessed with a heated pneumotachometer (model 3813; Hans Rudolph, Shawnee, KS). Peak expired flows, and flows at 75, 50 and 25% FVC were reported. An ethnic-neutral reference values from the Global Lung Function Initiative 2022 equations were used to determine %predicted spirometry values (286). Following the spirometry assessment, participants then performed a maximal cardiorespiratory exercise test on a cycle ergometer (LC7TT, Monark Exercise AB, Vansbro, Sweden). Detailed methods for the cardiorespiratory test can be found elsewhere (256). Briefly, participants completed a ramped

protocol starting at a resistance of 60 W, with increases of 20 $W \cdot min^{-1}$ until exhaustion. Measures of heart rate (HR) (Model T34; Polar Electro Oy, Kempele, Finland), mixed expired oxygen (O_2) and carbon dioxide (CO_2) (S-3-A/I and CD-3Am, Applied Electrochemistry, Bastrop, TX), and ventilation ($\dot{V}_E$) (model 3813; Hans Rudolph) were collected continuously, allowing for the determination of peak O_2 consumption during exercise ($\dot{V}O_{2peak}$).

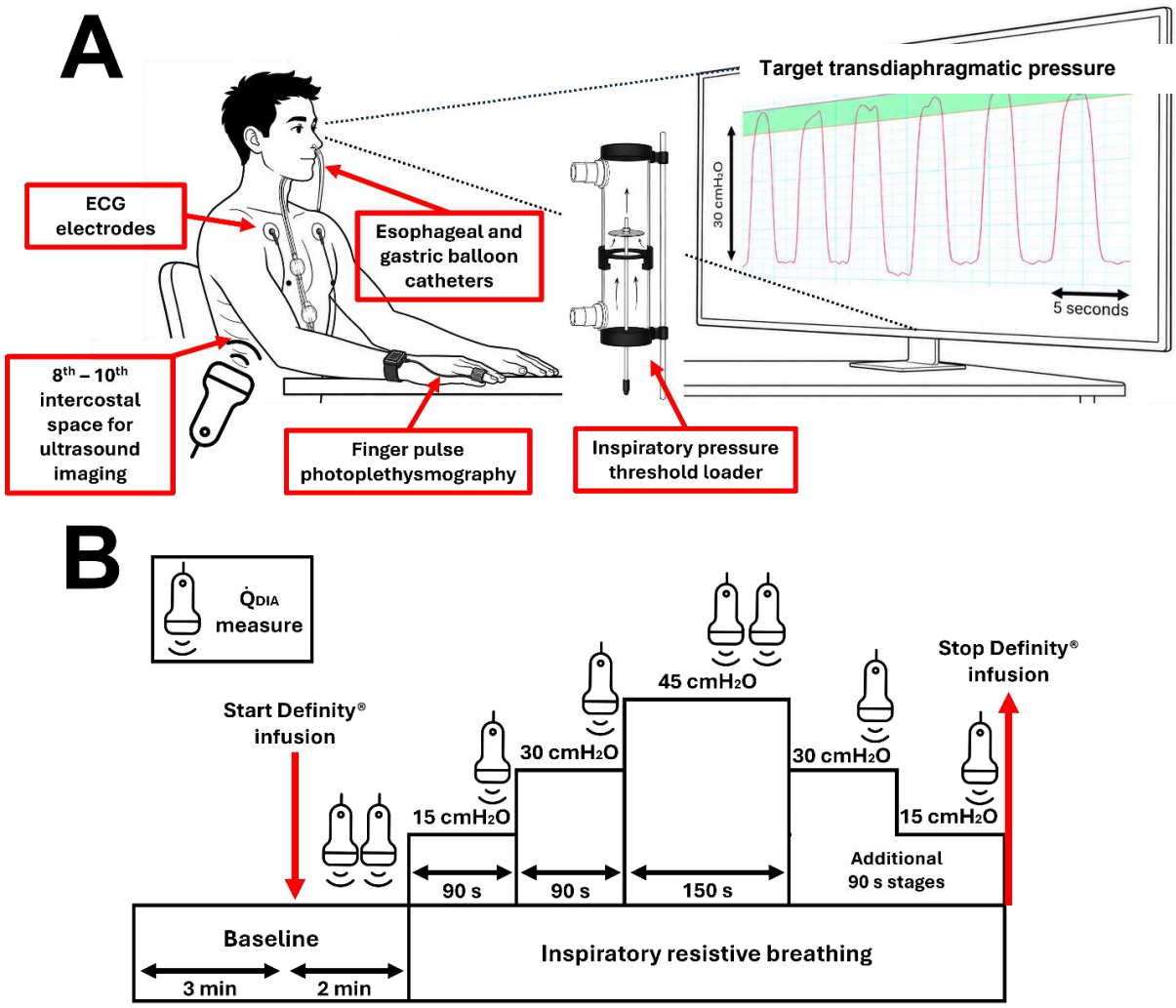


Figure 15. Representative schematic of cardiorespiratory instrumentation and experimental protocol

Representative schematic of the cardiorespiratory instrumentation and inspiratory threshold loading (ITL) protocol. Panel **A** shows electrocardiograph (ECG) electrodes, esophageal and gastric balloon catheters, ultrasound imaging, finger pulse photoplethysmography, and real-time diaphragm pressure visual feedback provided to the participant. Panel **B** outlines the inspiratory resistive breathing protocol.

ITL exercise protocol

After instrumentation, participants were seated in an upright position and measures of diaphragm thickness were taken at end-expiratory and end-inspiratory lung volumes during normal eupneic breathing. Afterwards, a series of maximal inspiratory efforts were performed to determine maximal mouth pressure (MIP) and P_{DIA} values (PDI_{MAX}). To determine maximal pressures, a minimum of three MIP efforts were performed where participants maximally inhaled. Afterwards a separate series of a minimum of three PDI_{MAX} efforts were performed whereby participants maximally inhaled with their stomach (targeting the diaphragm). For both MIP and PDI_{MAX} an average of three consistent efforts (<Δ5%) was reported. The participant then performed a brief warm up for two minutes breathing on the unloaded ITL. Upon completion of the warmup, participants remained on the ITL and began the exercise protocol depicted in Figure 15B. Participants completed 5 minutes of baseline, followed by 90 s stages targeting a P_{DIA} of 15 cmH_2O, 30 cmH_2O and 45 cmH_2O. Participants then performed an additional 45 cmH_2O stage for 60 s. Following completion of the initial ascending ramp protocol, in some participants two additional descending ramp stages were performed at the discretion of the researchers, targeting either a baseline, 15 or 30 cmH_2O stages. To ensure the time tension index of the diaphragm (TTI_{DIA}) did not exceed 0.3 and limit blood flow (287), absolute P_{DIA} targets of 15 , 30 and 45 cmH_2O were selected based on the predicted maximal inspiratory pressure of a representative female of average height at age 30 (288).

Cardiorespiratory measurements

Continuous measures of cardiorespiratory variables and respiratory flow were collected through the experimental visits. Heart rate was measured via 3 lead electrocardiogram (ePM 12M, Mindray Bio-Medical Electronics, Shenzhen, PR China) while continuous beat-to-beat blood

pressure was collected by finger plethysmography placed on the participants left hand (Human NIBP Nano Interface; ADInstruments, Colorado Springs, CO). Absolute measures of right arm brachial artery blood pressure were taken from an automated oscillometric sphygmomanometer at during the baseline condition (ePM 12M). Expired flow was measured by a heated pneumotachometer to 37°C (model 3813; Hans Rudolph) from which tidal volume, breathing frequency and $\dot{V}_E$ were derived. Tidal volume and $\dot{V}_E$ were expressed as body temperature saturated with water vapor at ambient pressure. Inspirated flow was similarly measured via pneumotachometer (model 3813, Hans Rudolph) and an inspiratory capacity maneuver was performed at the end of each exercise stage to determine lung volumes at the time of $\dot{Q}_{DIA}$ measurements. End-tidal O_2 and CO_2 concentrations were measured with calibrated gas analyzers (S-3-A/I and CD-3Am, Applied Electrochemistry, Bastrop, TX). Throughout the experimental session, CO_2 was titrated into the inspirate to maintain isocapnic conditions between baseline and exercise stages. Continuous measures of cardiorespiratory variables and respiratory flow was collected throughout the trial and ultrasound imaging of the diaphragm were made at the conclusion of each ITL stage.

Respiratory pressures

Respiratory pressures were measured at the mouth (P_M) from a sample port on the mouthpiece and from the placement of an esophageal (P_{ESO}) and gastric (P_{GAS}) balloon-tipped catheters (Guangzhou Respiratory Medical Science Co. Ltd, Guangzhou, China) connected to calibrated differential pressure transducers (DP15; Validyne Engineering, Northridge, CA, USA). Briefly, 2% lidocaine hydrochloride was provided to participants to anaesthetize the nares and throat before passing the balloon catheters through the nose and into the stomach. Participants performed a ~2 s Valsalva maneuver while catheters were open to the atmosphere to eliminate air

from the balloons. A glass syringe was connected to a 3-way Luer lock of the balloon catheters and 0.5 mL of air was placed in the esophageal balloon and 1.2 mL of air filled the gastric balloon. Verification of balloon placement was performed using the dynamic occlusion test, confirming the ratio between the change in P_{ESO} and P_M was ~1 before securing the balloon catheters to the participants face with tape (289).

Transdiaphragmatic pressure (P_{DIA}) was calculated as the difference between P_{GAS} and P_{ESO}. The pressure-time product of P_{DIA} (PTP_{DIA}) was calculated as the product of average inspiratory pressure and the duration of inspiration and was used as an index of diaphragm muscle work. The tension-time index of the diaphragm (TTI_{DIA}), relating the force developed by the diaphragm to the time it is active was calculated with the following equation:

$$(7) \quad TTI_{DIA} = \frac{P_{DIA}}{P_{DIA,max}} \times DC$$

Where P_{DIA} is the mean inspiratory transdiaphragmatic pressure, PDI_{MAX} is the maximal transdiaphragmatic pressure generated by the participant during a MIP manoeuvre and DC is respiratory the duty cycle (290).

Diaphragm imaging and contrast enhanced ultrasound

Diaphragm measurements were made using ultrasound imaging (GE Logiq E9 Ultrasound; GE Healthcare, Chicago, IL) with a linear probe (GE 9L-D) to identify the costal diaphragm, external obliques, intercostal muscles and liver (291). Measures of diaphragm morphology were made prior to each experimental visit. Measures of diaphragm thickness were performed at end-expiration and end-inspiration during normal tidal breathing. The change in tidal thickness was the difference between end-expiration and end-inspiration diaphragm thickness and the percent tidal

thickening fraction was calculated as the quotient of the change in tidal thickness over end-inspiration diaphragm thickness multiplied by 100.

Blood flow measurements of the diaphragm were performed using contrast enhanced ultrasound imaging (CEUS) at the end of baseline and exercise stages (111, 137). Briefly, a 35 ml syringe was inserted into an infusion pump (Baxter AS50 Infusion Pump, Baxter International Inc., Deerfield, IL, USA) and connected to microbore tubing feeding into a 22-gauge catheter placed in the antecubital vein of the participant. Within the syringe was a lipid-encapsulated perfluoropropane microbubble contrast enhancing imaging agent (*Definity*®, Lantheus Medical Imaging, North Billerica, Mass) diluted 1:20 in normal saline. Two minutes before the end of the baseline condition, the *Definity*® infusion was initiated and administered at a rate of 2.5 ml/min until completion (~12 minutes).

The contrast agent was visualized in the diaphragm via B-mode ultrasound images recorded on a single ultrasound machine (GE Logiq E9 Ultrasound). To acquire consistent images of the diaphragm, an ultrasound transducer was placed between the 8^{th} – 10^{th} intercostal space and participants performed a 10 s apnea following a normal expiration (i.e., at functional residual capacity). Twenty second B-mode ultrasound recordings of the diaphragm were collected at a frame rate of 11 Hz and ultrasound frequency of 4 MHz, dynamic range of 63, and mechanical index ranging from 0.09-0.12. Destruction of the microbubble contrast agent was performed with the "Flash" function on the ultrasound, emitting a short ultrasound wave at a high mechanical index of 1.2, bursting all the microbubbles located within the area of interest. The time after the elimination of the microbubble contrast agents for each image is termed the pulsing interval (PI) (111, 148, 155). Video intensity (VI) was determined from the identified regions of interest,

providing a measure of the contrast agent density within the microvasculature (111, 148, 155). Blood flow was then be determined by fitting PI vs VI to equation (2):

$$y = A(1 - e^{-Bt})$$

Where y is the VI at time t and A is the plateau intensity, reflecting microvascular blood volume (MBV) of the region of interest in arbitrary units (AU), B is the rate constant of microbubble replenishment and is a proportional to the microvascular blood flux rate (MFR) of the tissue in s^{-1} (111, 148, 155). A measure of $\dot{Q}_{DIA}$ may then be determined as the product of A and B. All CEUS examinations and analysis was performed by the same ultrasound sonographer (C.J.D.).

Propranolol administration

Beta-adrenergic receptor blockade was achieved by having participants ingest two oral doses of 80 mg propranolol HCl (160 mg total) prior to their PROP visit. The first dose was administered 7 hours before the visit, while the second dose was administered 1 hour before the start of experimental testing to ensure peak blood propranolol concentrations coincided with the testing visit. A two-dose regimen was chosen to help mitigate the effects of first-pass liver metabolism before administration of the second dose (274). Importantly, male and female participants in the present study showed no difference in the reduction in resting heart rate with propranolol (-Δ27 ± 14% vs. -Δ27 ± 13%, p = 0.92; males and females, respectively), which is consistent with the reductions in resting heart rate previously reported during complete beta-adrenergic blockade (range: ~-Δ15–25%) (8, 246, 276). Therefore, we are confident that the propranolol dosing regimen was sufficient to achieve complete β-AR blockade across all participants.

Data Analysis

All continuous measures of cardiovascular variables were collected at 200 Hz on a 16 channel Powerlab (ADInstruments, Colorado Springs, CO, USA) and reported as a beat-by-beat average over the final 30 s of each stage. Measures of respiratory pressures (P_{ESO}, P_{GAS}, P_{DIA}) were reported as a breath-by-breath average of the inspiratory phase over the final 30 s average of each stage. Given the lowest P_{DIA} value during the expiratory phase varied between individuals from ~5-21 cmH_2O (mean, 12.44 ± 3.7cmH_2O), the reported inspiratory P_{DIA} pressure was calculated as the difference between inspiratory P_{DIA} and expiratory P_{DIA} values. The work performed by the diaphragm (i.e., pressure-time product) was calculated by integrating P_{DIA} during the inspiration phase for the final 30 s of each stage. TTI_{DIA} was calculated as the product of P_{DIA}/PDI_{MAX} and inspiratory phase time/total respiratory cycle time.

Figure 16 outlines the destruction-replenishment curves generated for both control and propranolol conditions and representative ultrasound images visualizing contrast microbubbles in the diaphragm during a collection sequence. Contrast enhanced ultrasound cineloops were analyzed using specialized software (narnar app; narnar, Lake Oswego, OR, USA). Using this software, video visual intensity was quantified from the diaphragm on a frame-by-frame basis. Time and acoustic intensity were determined from the region of interest (ROI) within the diaphragm and fitted to the destruction-replenishment. This analysis technique has been previously used to characterize microvascular perfusion of the diaphragm (111, 137). Measures of diaphragm vascular conductance (DVC) were calculated as the quotient of $\dot{Q}_{DIA}$ and MAP ($DVC_{\dot{Q}DIA}$) or MFR and MAP (DVC_{MFR}), respectively. Relative P_{DIA} values were reported as a percentage of PDI_{MAX} for each individual.

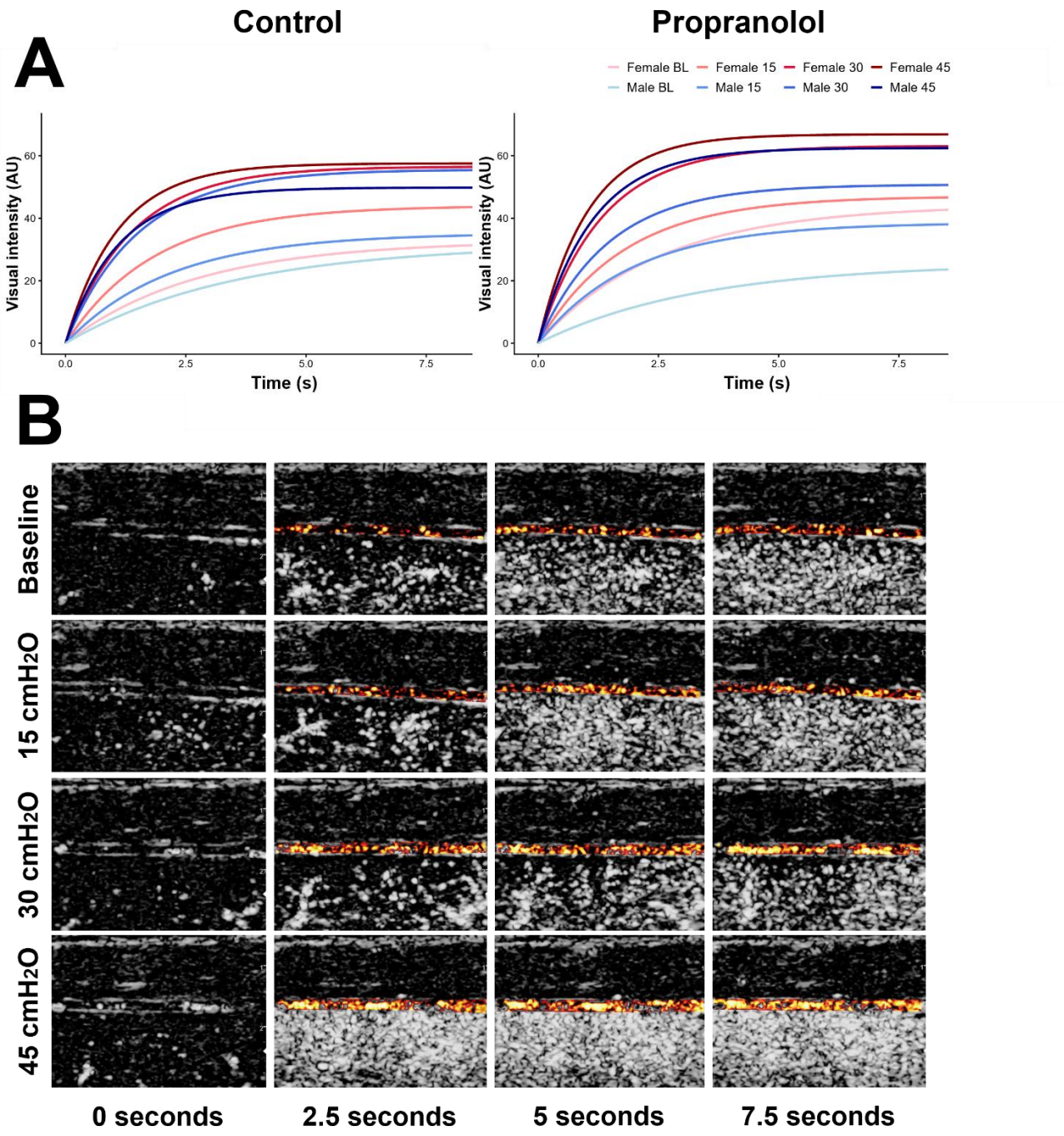


Figure 16. Mean destruction-replenishment curves and representative visualization of contrast agent in the diaphragm

Destruction-replenishment curves for the control and propranolol conditions for diaphragm blood flow and representative visualization of microbubbles in the diaphragm. Panel **A** outlines the average time-intensity data from the diaphragm during baseline and progressive diaphragm pressures. Panel **B** is a representative image of ultrasound imaging to visualize contrast microbubbles accumulating in the diaphragm of participant during diaphragm blood flow assessment.

Statistical analysis

Statistical analysis for Student's *t*-test, 2-way ANOVA, 3-way ANOVA, and simple linear regressions were performed using GraphPad Prism Software (GraphPad Prism 11.0.1, LaJolla, CA). Sex differences in participant characteristics, spirometry assessments and peak exercise values were assessed using a using a Student's *t*-test. Beat-by-beat cardiorespiratory variables, respiratory pressures and diaphragm perfusion measures were analyzed using a 3-way ANOVA (sex x condition x exercise intensity). Of note no 3-way interaction was observed for any variable (all; $P > 0.05$) and was thus not reported in the results section. A 2-way ANOVA (sex x condition) was performed to examine baseline cardiorespiratory, respiratory pressures, and diaphragm measures. Significant main effects are interactions were probed using Tukey's post-hoc testing to assess differences in mean responses. To assess whether $\dot{Q}_{DIA}$ measures taken during the ascending ramp were different from measures taken during the descending ramp, a simple linear regression analysis was performed examining the relationship between MFR and diaphragm work. To perform this analysis, propranolol and control conditions were combined across all participants and only instances where baseline and exercise stages that were both performed during the ramp and following the first 45 cmH_2O stage were included. There were 18 measures for baseline, and 11, 7, and 20 measures included for the 15, 30, and 45 cmH_2O stages, respectively. Linear mixed model analysis and figure generation was performed using R (v4.3.1) to examine the relationship between diaphragm perfusion ($\dot{Q}_{DIA}$, MFR, $DVC_{\dot{Q}DIA}$, DVC_{MFR}) and diaphragm effort (PTP_{DIA}, P_{DIA}, P_{DIA} relative to PDI_{MAX}). Where diaphragm effort was the continuous predictor, participant as a random intercept, while sex and condition were fixed effects. Interaction terms between diaphragm efforts, sex and condition were included to assess whether the relationship between

diaphragm effort and diaphragm perfusion differed by sex or condition. The acceptable Type I error rate was set *a priori* at $\alpha = 0.05$.

5.3 Results

Participant characteristics

Participant characteristics and spirometry and peak cardiorespiratory exercise variables can be found in Table 9. Males were taller and had greater body mass compared to females (both $P < 0.01$). Males were also found to have greater absolute FVC and FEV_1 (both < 0.01); however, percent predicted values were not different by sex (both $P > 0.05$). All other spirometry measures were not different between sexes (all $P > 0.05$). Males achieved a greater peak power output and ventilation compared to females (both $P < 0.03$). Absolute $\dot{V}O_{2peak}$ and $\dot{V}CO_{2peak}$ values were also greater in males (both $P < 0.01$), whereas relative $\dot{V}O_{2peak}$ normalized to body mass was not different between sexes ($P = 0.56$). Peak exercise heart rate and respiratory exchange ratio values at peak exercise were comparable between sexes, indicating a similar maximal exercise effort was achieved.

Table 9. Chapter 5 participant characteristics, spirometry assessments and peak exercise test variables

Participant characteristics	Males (n=7)	Females (n =7)	*P*-value
Age (y)	28 ± 6	27 ± 6	0.86
Height (m)	1.80 ± 0.07	1.65 ± 0.03	<0.01
Body mass (kg)	76.4 ± 11.0	63.6 ± 3.5	0.01
Body mass index ($kg \cdot m^2$)	23.5 ± 2.4	23.5 ± 1.9	0.98
Spirometry			
FVC (L)	5.61 ± 0.78	3.81 ± 0.50	<0.01
(% pred)	(107 ± 19)	(105 ± 12)	(0.74)
FEV_1	4.58 ± 0.71	3.35 ± 0.49	<0.01
(% pred)	(105 ± 19)	(107 ± 15)	(0.80)
FEV_1/FVC (%)	0.82 ± 0.08	0.88 ± 0.06	0.11
(% pred)	(97 ± 8)	(102 ± 7)	0.32
Peak expired flow ($L \cdot s^{-1}$)	11.1 ± 1.9	8.1 ± 1.9	0.01
Flow at 75% ($L \cdot s^{-1}$)	9.0 ± 1.6	7.3 ± 1.8	0.08
Flow at 50% ($L \cdot s^{-1}$)	5.7 ± 1.4	5.5 ± 1.8	0.86
Flow at 25% ($L \cdot s^{-1}$)	2.5 ± 0.6	2.7 ± 0.8	0.65
MIP (cmH_2O)	151 ± 25	138 ± 37	0.48
PDI_{MAX} (cmH_2O)	170 ± 24	168 ± 30	0.93
Peak exercise			
Power output (W)	299 ± 32	242 ± 40	0.01
Heart rate (bpm)	184 ± 8	184 ± 7	0.99
Ventilation (L/min)	175 ± 20	142 ± 29	0.03
Absolute VO_{2peak} ($L \cdot min^{-1}$)	3.91 ± 0.56	3.10 ± 0.49	0.01
Relative VO_{2peak} ($mL \cdot min^{-1} \cdot kg^{-1}$)	51.6 ± 6.8	49.0 ± 9.0	0.56
VCO_{2peak} ($L \cdot min^{-1}$)	4.24 ± 0.37	3.32 ± 0.37	<0.01
Respiratory exchange ratio	1.08 ± 0.07	1.09 ± 0.07	0.75

Values are mean ± SD. FVC, forced vital capacity; FEV_1, forced expired volume in 1 second; MIP, maximal inspiratory pressure from the mouth; PDI_{MAX}, maximal transdiaphragmatic pressure. VO_{2peak}, peak oxygen uptake; VCO_{2peak}, peak carbon dioxide produced.

Resting measures

Baseline resting cardiorespiratory, respiratory pressure, diaphragm morphology, and diaphragm perfusion variables for control and propranolol conditions are presented in Table 10. A main effect of propranolol was observed for resting heart rate and MAP (both, $P < 0.05$), with lower values present during propranolol compared to control. A main effect of sex was observed for tidal volume and $\dot{V}_E$ (both $P < 0.05$), with males demonstrating greater values compared to

females. Breathing frequency was not different between sex or condition groups (both $P > 0.05$). No main effects of sex or propranolol were observed for respiratory pressure variables, including P_M, P_{ESO}, P_{GAS} P_{DIA}, TTI_{DIA}, or PTP_{DIA} (all $P > 0.05$).

Table 10. Resting baseline cardiorespiratory variables in males and females.

	Control		Propranolol	
Variable	Male	Female	Male	Female
Cardiorespiratory	n=7	n=7	n=7	n=7
Heart rate (bpm) †	79 ± 12	73 ± 12	62 ± 4	57 ± 7
MAP (mmHg) †	88 ± 13	83 ± 7	84 ± 11	79 ± 7
Tidal volume (L) *	1.4 ± 0.3	1.0 ± 0.2	1.6 ± 0.9	1.0 ± 0.4
Breathing frequency (breath·min^{-1})	14 ± 1	15 ± 1	14 ± 1	15 ± 1
Ventilation (L·min^{-1}) *	19.3 ± 4.6	13.9 ± 2.3	21.4 ± 10.1	14.5 ± 5.7
Respiratory pressures				
P_M (cmH_2O)	-1.8 ± 0.4	-1.7 ± 0.7	-1.7 ± 0.3	-1.3 ± 0.4
P_{ESO} (cmH_2O)	-3.5 ± 1.2	-3.2 ± 1.2	-3.9 ± 3.3	-3.0 ± 1.2
P_{GAS} (cmH_2O)	2.5 ± 2.2	2.8 ± 2.6	2.4 ± 2.6	3.1 ± 1.6
P_{DIA} (cmH_2O)	5.9 ± 3.3	6.1 ± 3.0	6.3 ± 3.3	6.1 ± 1.9
TTI_{DIA}	0.05 ± 0.02	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01
PTP_{DIA} ($cmH_2O \cdot s^{-1}$)	12.0 ± 6.3	11.6 ± 6.4	10.7 ± 4.9	11.0 ± 2.8
Diaphragm variables				
End-expiration thickness (mm)	1.7 ± 0.3	1.5 ± 0.3	1.7 ± 0.3	1.5 ± 0.3
End-inspiration thickness (mm)	3.0 ± 0.8	2.5 ± 0.6	3.0 ± 1.0	2.5 ± 0.6
ΔTidal thickness (mm)	1.3 ± 0.6	1.0 ± 0.4	1.3 ± 0.7	1.0 ± 0.4
Tidal thickening fraction (%)	42 ± 10	38 ± 7	41 ± 11	39 ± 8
Diaphragm perfusion				
Microvascular flux rate (s^{-1})	0.29 ± 0.10	0.37 ± 0.08	0.30 ± 0.10	0.39 ± 0.09
Microvascular blood volume (AU)	32 ± 24	33 ± 12	26 ± 17	44 ± 23
$\dot{Q}_{DIA}$ (AU·s^{-1}) *	8.0 ± 4.1	11.7 ± 4.3	7.0 ± 3.0	16.9 ± 11.9
DVC_{MFR} ($s^{-1} \cdot mmHg^{-1} \cdot 100$)	0.34 ± 0.10	0.45 ± 0.10	0.38 ± 0.18	0.48 ± 0.09
$DVC_{\dot{Q}DIA}$ (AU·s^{-1}·mmHg^{-1}) *	0.10 ± 0.07	0.15 ± 0.05	0.09 ± 0.03	0.20 ± 0.09

Values are means ± SD. MAP, mean arterial pressure; P_M, inspiratory mouth pressure; P_{ESO}, inspiratory esophageal pressure; P_{GAS}, inspiratory gastric pressure; P_{DIA}, inspiratory transdiaphragmatic pressure; TTI_{DIA}, tension-time index of the diaphragm; PTP_{DIA}, pressure time product of the diaphragm; ΔTidal thickeness, change in diaphragm thickness during tidal breathing; $\dot{Q}_{DIA}$, diaphragm blood flow; DVC_{MFR}, diaphragm vascular conductance from microvascular flus rate; $DVC_{\dot{Q}DIA}$, diaphragm vascular conductance from diaphragm blood flow. *$P < 0.05$ main effect of sex; †$P < 0.05$ main effect of propranolol. No significant sex × condition interaction was observed.

Diaphragm morphology variables, including end-expiration thickness, end-inspiration thickness, the change in tidal thickness, and tidal thickening fraction, were not different between sex or propranolol conditions (all $P > 0.05$). For diaphragm perfusion variables, a main effect of sex was observed for $\dot{Q}_{DIA}$ and $DVC_{\dot{Q}DIA}$ (both $P < 0.05$), with females demonstrating greater values compared to males. Microvascular flux rate, microvascular blood volume, and DVC_{MFR} were not different between sex or propranolol conditions (all $P > 0.05$). No sex × condition interactions were observed for any baseline variable.

Respiratory pressures and cardiorespiratory variables during threshold loading

Respiratory pressures and diaphragm effort responses to incremental inspiratory threshold loading exercise are found in Table 11. A significant main effect exercise intensity was observed across all respiratory pressure and diaphragm effort variables (all $P < 0.05$), with values increases from baseline to the 45 cmH_2O stage. No main effects of sex or condition was observed for any variable, and no significant interactions were present (all $P > 0.05$).

Cardiorespiratory responses to increasing inspiratory threshold loading are presented in Figure 17. Heart rate increased with target P_{DIA} pressure ($P < 0.01$) and was lower during propranolol compared to control conditions ($P < 0.01$), with no main effect of sex ($P = 0.25$). MAP was increased with P_{DIA} pressures and was lower during the propranolol condition (both $P < 0.05$), but no sex difference was observed ($P > 0.05$). Ventilation increased with increases in P_{DIA} pressure ($P < 0.01$) but no main effect of condition or sex was observed (both $P > 0.05$). End-tidal CO_2 demonstrated a modest effect of P_{DIA} pressure ($P = 0.03$), but no main effects of condition or sex were observed (both $P > 0.05$). No additional interactions between sex, condition were observed for any cardiorespiratory variable (all $P > 0.05$).

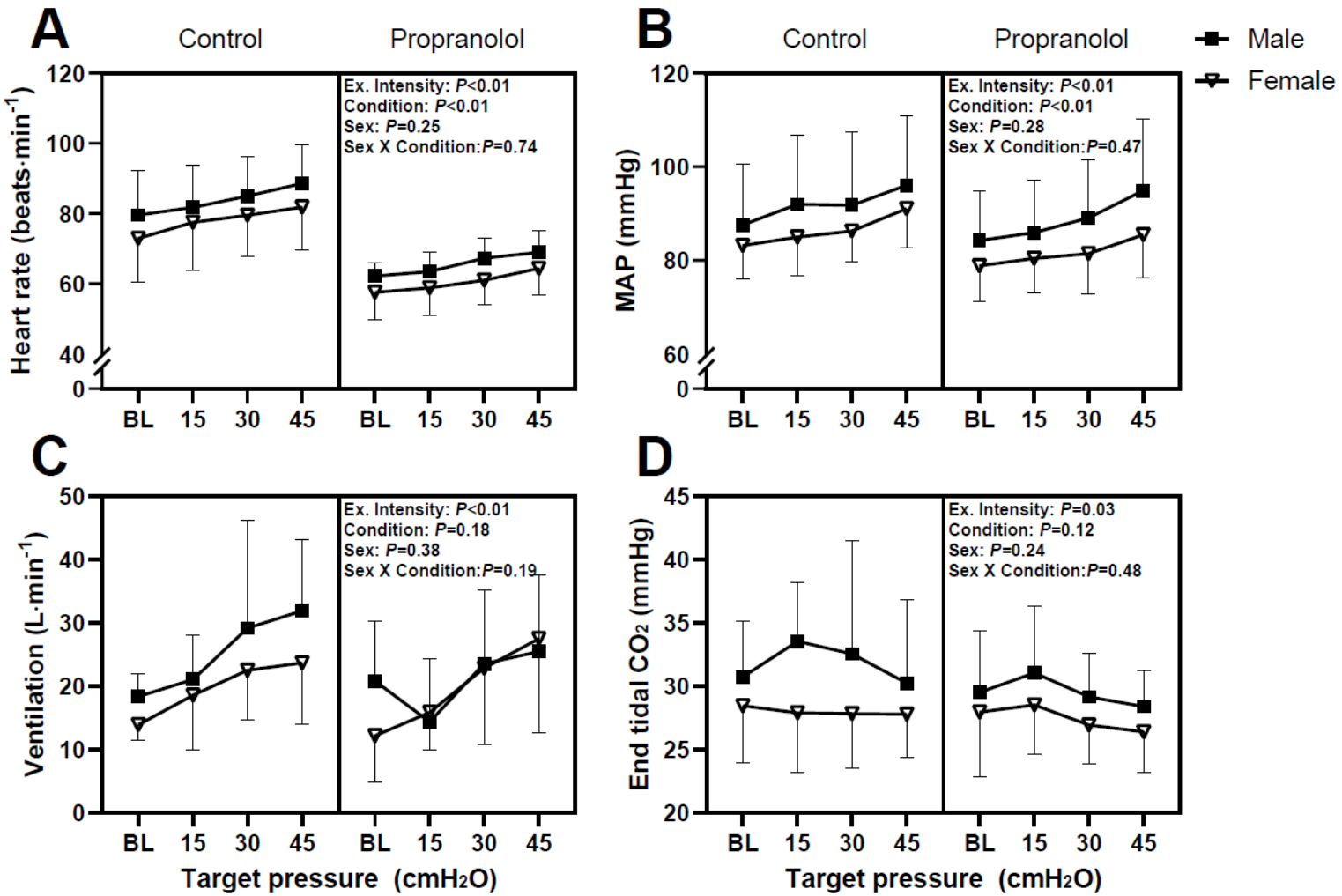


Figure 17. Heart rate, mean arterial pressure, ventilatory and end-tidal carbon dioxide measures during inspiratory threshold loading.

Absolute heart rate (**A**), mean arterial pressure (MAP) (**B**), ventilation (**C**), and end tidal carbon dioxide (CO_2) (**D**) during baseline and diaphragm target pressures of 15, 30, and 45 cmH_2O. Males denoted by black squares and females denoted by open triangles, respectively.

Table 11. Mean inspiratory respiratory pressures, diaphragm efforts and inspiratory capacities during incremental inspiratory threshold loading.

		Target transdiaphragmatic pressure (cmH_2O)			
Control	Sex	Baseline	15	30	45
P_M (cmH_2O)	Male	-1.7 ± 0.3	-8.3 ± 0.9	-10.3 ± 1.1	-13.4 ± 2.8
	Female	-1.7 ± 0.7	-7.1 ± 3.0	-9.9 ± 2.6	-13.1 ± 3.7
%MIP (%)	Male	1.2 ± 0.4	5.8 ± 1.6	7.3 ± 2.1	9.5 ± 3.7
	Female	1.3 ± 0.6	5.4 ± 2.6	7.5 ± 2.5	10.0 ± 3.6
P_{ESO} (cmH_2O)	Male	-3.5 ± 1.2	-9.8 ± 2.6	-13.3 ± 4.6	-17.4 ± 6.5
	Female	-3.2 ± 1.2	-10.8 ± 4.0	-13.3 ± 2.5	-16.6 ± 2.8
P_{GAS} (cmH_2O)	Male	2.5 ± 2.2	2.1 ± 2.1	6.6 ± 5.6	12.7 ± 11.5
	Female	2.8 ± 2.6	2.2 ± 2.5	6.2 ± 4.0	12.2 ± 5.0
P_{DIA} (cmH_2O)	Male	5.9 ± 3.3	12.0 ± 1.1	20.0 ± 1.5	30.1 ± 5.1
	Female	6.1 ± 3.0	13.0 ± 1.9	19.5 ± 2.9	28.8 ± 4.0
P_{DIA} swings	Male	10.6 ± 4.4	19.3 ± 2.6	34.1 ± 2.7	49.5 ± 4.8
(cmH_2O)	Female	11.0 ± 4.2	20.3 ± 1.9	34.1 ± 4.1	50.5 ± 4.1
$\%PDI_{MAX}$ (%)	Male	3.5 ± 1.9	6.7 ± 1.4	12.0 ± 1.8	17.9 ± 3.0
	Female	3.6 ± 1.6	7.2 ± 1.0	11.8 ± 2.2	17.5 ± 3.9
TTI_{DIA}	Male	0.02 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.11 ± 0.02
	Female	0.02 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.10 ± 0.02
PTP_{DIA}	Male	12.0 ± 6.3	27.4 ± 6.0	50.2 ± 8.6	77.5 ± 20.0
($cmH_2O \cdot s$)	Female	11.6 ± 6.4	25.5 ± 4.2	45.8 ± 8.5	63.8 ± 12.2
Inspiratory	Male	2.7 ± 0.7	3.0 ± 0.6	3.5 ± 0.7	3.5 ± 0.8
capacity (L) *	Female	1.7 ± 0.3	2.1 ± 0.4	2.1 ± 0.5	2.3 ± 0.5

		Target transdiaphragmatic pressure (cmH_2O)			
Propranolol	Sex	Baseline	15	30	45
P_M (cmH_2O)	Male	-1.6 ± 0.3	-7.3 ± 0.8	-9.6 ± 1.0	-12.3 ± 2.5
	Female	-1.3 ± 0.4	-6.1 ± 3.0	-10.3 ± 2.5	-13.1 ± 3.2
%MIP (%)	Male	1.2 ± 0.4	5.1 ± 1.4	6.7 ± 1.8	8.7 ± 3.2
	Female	1.0 ± 0.4	4.8 ± 2.5	7.9 ± 2.7	10.1 ± 3.6
P_{ESO} (cmH_2O)	Male	-3.9 ± 3.3	-7.7 ± 0.9	-11.9 ± 3.3	-16.3 ± 6.3
	Female	-3.0 ± 1.2	-9.1 ± 4.0	-13.2 ± 4.0	-18.2 ± 3.0
P_{GAS} (cmH_2O)	Male	2.4 ± 2.6	5.3 ± 1.7	9.3 ± 3.3	14.5 ± 8.7
	Female	3.1 ± 1.6	4.1 ± 3.5	8.2 ± 5.2	12.8 ± 4.5
P_{DIA} (cmH_2O)	Male	6.3 ± 3.3	13.0 ± 2.1	21.2 ± 2.6	30.8 ± 6.3
	Female	6.1 ± 1.9	13.3 ± 1.6	21.4 ± 2.4	31.0 ± 3.4
P_{DIA} swings	Male	10.1 ± 3.8	20.6 ± 2.8	34.5 ± 3.8	50.9 ± 4.9
(cmH_2O)	Female	10.9 ± 2.5	20.3 ± 2.1	37.6 ± 3.6	52.9 ± 5.1
$\%PDI_{MAX}$ (%)	Male	3.1 ± 1.9	7.9 ± 1.8	12.6 ± 1.6	18.2 ± 2.9
	Female	3.6 ± 0.9	7.7 ± 1.6	13.0 ± 2.2	18.9 ± 3.6
TTI_{DIA}	Male	0.02 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.11 ± 0.02
	Female	0.02 ± 0.01	0.05 ± 0.01	0.08 ± 0.02	0.11 ± 0.02
PTP_{DIA}	Male	10.7 ± 4.9	31.3 ± 3.4	50.9 ± 9.8	73.7 ± 19.9
($cmH_2O \cdot s^{-1}$)	Female	11.0 ± 2.8	26.9 ± 6.1	49.8 ± 9.3	71.7 ± 9.0

Inspiratory capacity (L)	Male	2.7 ± 0.5	3.0 ± 0.4	3.2 ± 0.6	3.3 ± 0.6
	Female	1.7 ± 0.3	2.0 ± 0.4	2.2 ± 0.5	2.3 ± 0.5

Values are means ± SD. P_M, inspiratory mouth pressure; %MIP, mouth pressure relative to maximal inspiratory pressure; P_{ESO}, inspiratory esophageal pressure; P_{GAS}, inspiratory gastric pressure; P_{DIA}, inspiratory transdiaphragmatic pressure; $\%PDI_{MAX}$ TTI_{DIA}, tension-time index of the diaphragm; PTP_{DIA}, pressure time product of the diaphragm. A significant main effect of exercise intensity ($P < 0.01$) was observed for all variables. **$P < 0.05$ main effect of sex. No main effect of condition or sex ×condition was observed.

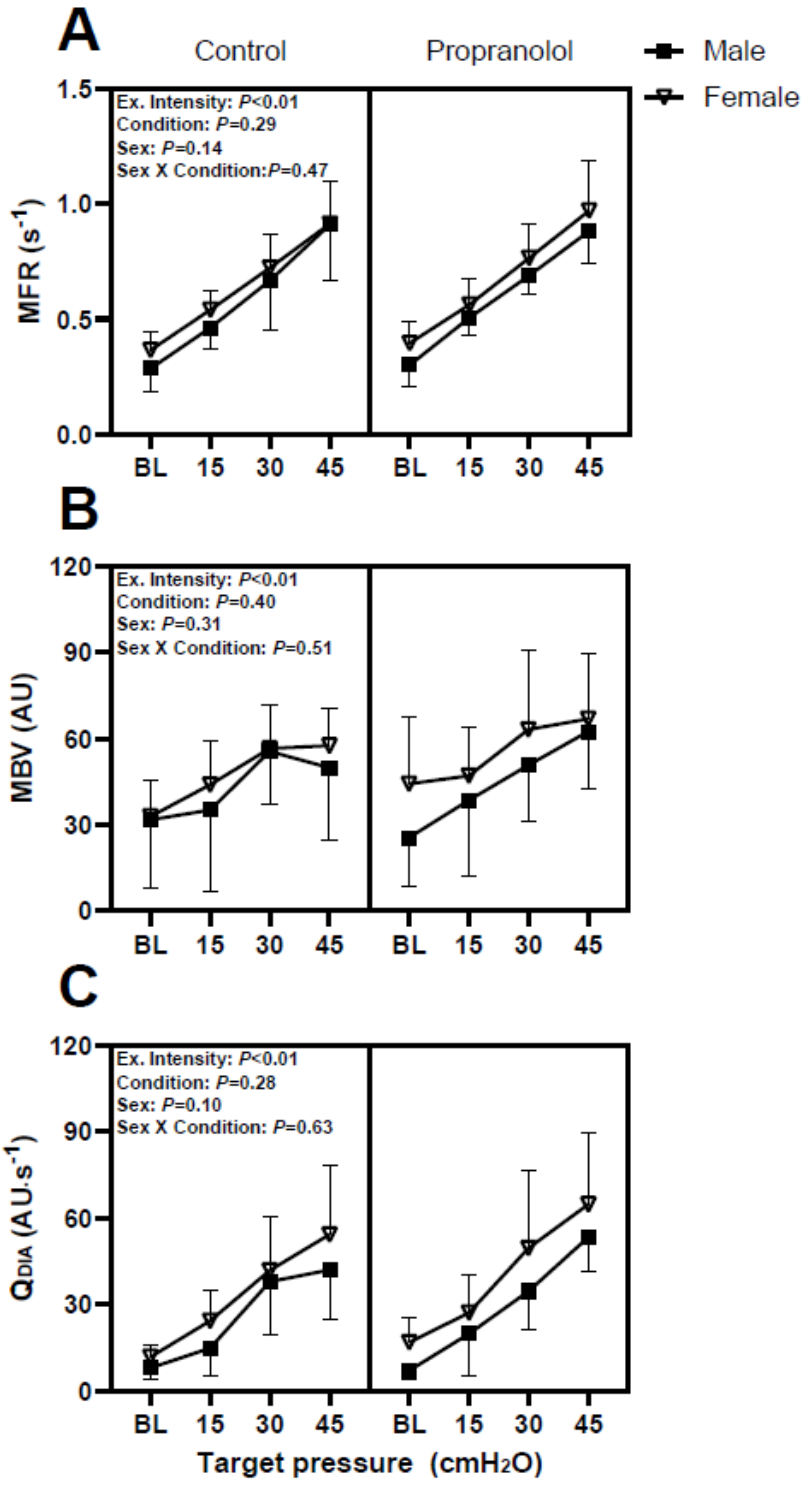


Figure 18. Diaphragm microvascular blood flow measures during inspiratory threshold loading

Diaphragm microvascular flux rate (MFR) (**A**), microvascular blood volume (MBV) (**B**), and diaphragm blood flow ($\dot{Q}_{DIA}$) (**C**) during baseline and diaphragm target pressures of 15, 30 and 45 cmH_2O. Males denoted by black squares and females denoted by open triangles, respectively.

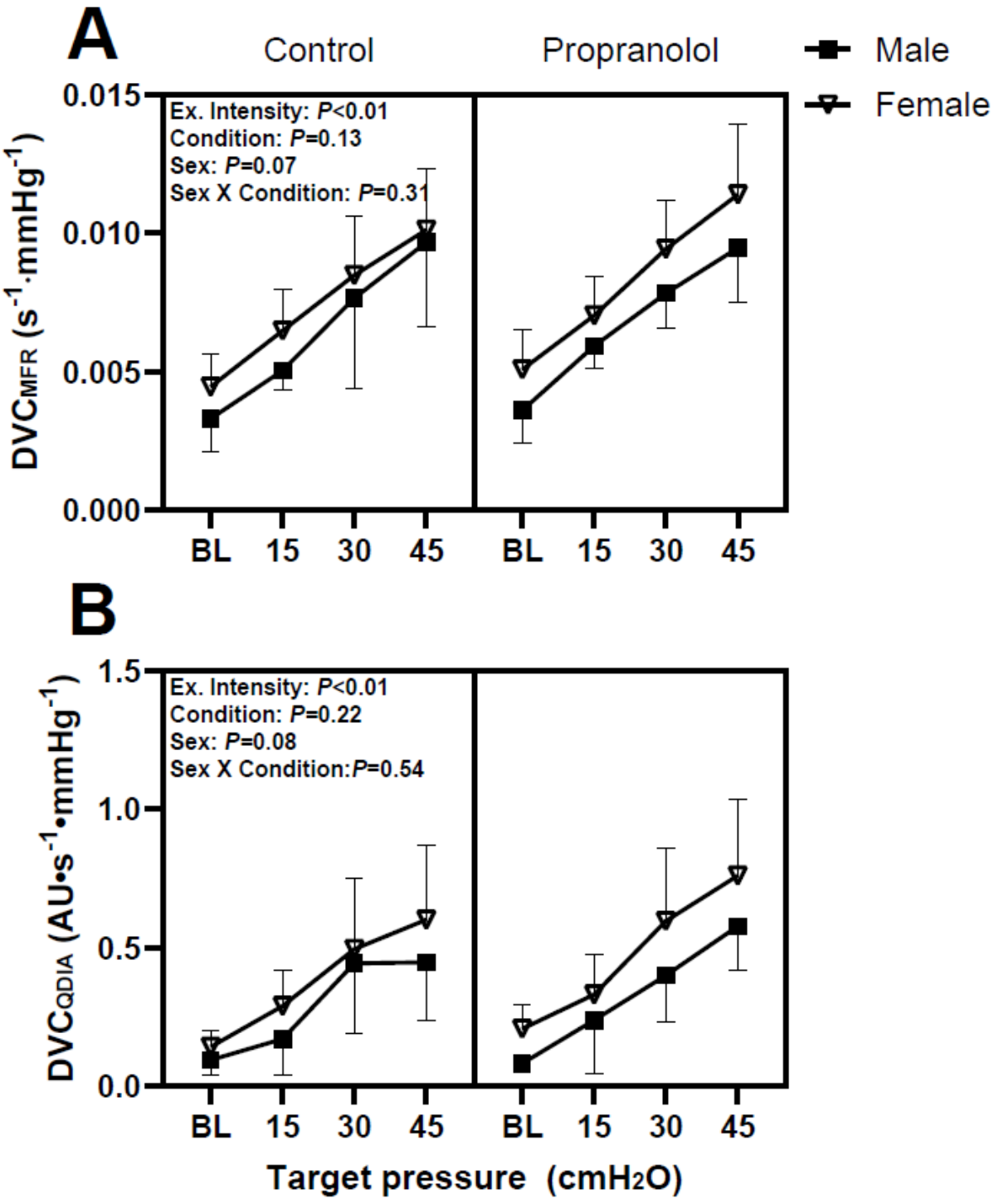


Figure 19. Diaphragm vascular conductance during inspiratory threshold loading.

Diaphragm vascular conductance from microvascular flux rate (DVC_{MFR}) (**A**) and diaphragm vascular conductance from diaphragm blood flow (DVC_{QDIA}) (**B**), during baseline and diaphragm target pressures of 15, 30 and 45 cmH_2O. Males denoted by black squares and females denoted by open triangles, respectively.

Diaphragm blood flow and vascular conductance during threshold loading

Mean diaphragm microvascular blood flow responses to incremental inspiratory loading are presented in Figure 18. MFR, MBV, and $\dot{Q}_{DIA}$ increased with greater P_{DIA} (all, P <0.01). There was no main effect of condition, sex and no interaction for all measures of $\dot{Q}_{DIA}$ (all, P > 0.05). Figure 19 outlines the diaphragm vascular conductance responses to incremental diaphragm target pressures. DVC_{MFR} and $DVC_{\dot{Q}DIA}$ increased significantly with exercise intensity (both, P <0.01). However, no main effects of condition or sex and no interaction was observed for either variable (all, P > 0.05). Figure 20 is a comparison of simple linear regression curves between the relationship of MFR, and diaphragm work of measures taken during the initial ascending ramp protocol versus additional measures of taken during the descending ramp. There was no difference in slope between measures taken during ramp protocol or post-ramp (P = 0.59).

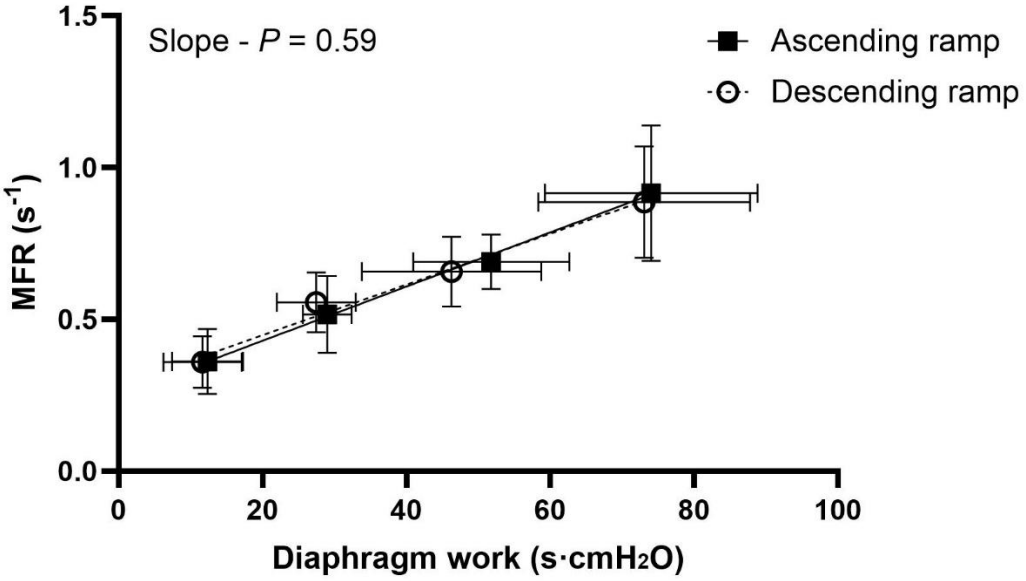


Figure 20. Linear regression of diaphragm microvascular flux rate and diaphragm work taken during the initial ascending ramp versus additional measurements during the descending ramp.

Simple linear regression of diaphragm microvascular flux rate (MFR) and diaphragm work for contrast enhanced diaphragm perfusion measurements taken during the initial ascending ramp (solid square, solid line) versus additional measurements taken during the descending ramp(open circle, dotted line).

Linear mixed model analysis of diaphragm MFR, $\dot{Q}_{DIA}$, DVC_{MFR} and $DVC_{\dot{Q}DIA}$ over diaphragm work can be found in Figure 21. Diaphragm microvascular blood flow and vascular conductance increased along with increases in diaphragm work. However, sex, condition, and sex x condition were not found to impact the slope between MFR, QDIA, DVC_{MFR} and $DVC_{\dot{Q}DIA}$ over diaphragm work (all $P > 0.05$). Figure 22 outlines the linear mixed model analysis of diaphragm blood flow and vascular conductance over P_{DIA}. Again, the slope of MFR, $\dot{Q}_{DIA}$, DVC_{MFR} and $DVC_{\dot{Q}DIA}$ over P_{DIA} was not found to be different by sex, condition or the interaction of sex x condition (all $P > 0.05$). Figure 23 displays the linear mixed model of diaphragm microvascular blood flow and vascular conductance over P_{DIA} relative to PDI_{MAX}. The slopes of MFR, $\dot{Q}_{DIA}$, DVC_{MFR} and $DVC_{\dot{Q}DIA}$ over increases in relative P_{DIA} were not impacted by sex or condition (all $P > 0.05$). Interestingly a significant sex × condition interaction was observed, indicating the effect of propranolol on the DVC_{MFR} versus relative P_{DIA} differed between sexes. Indeed, post hoc comparisons within sex found females (both $P = 0.06$) but not males (both $P = 0.15$) tended to have a greater slope with propranolol.

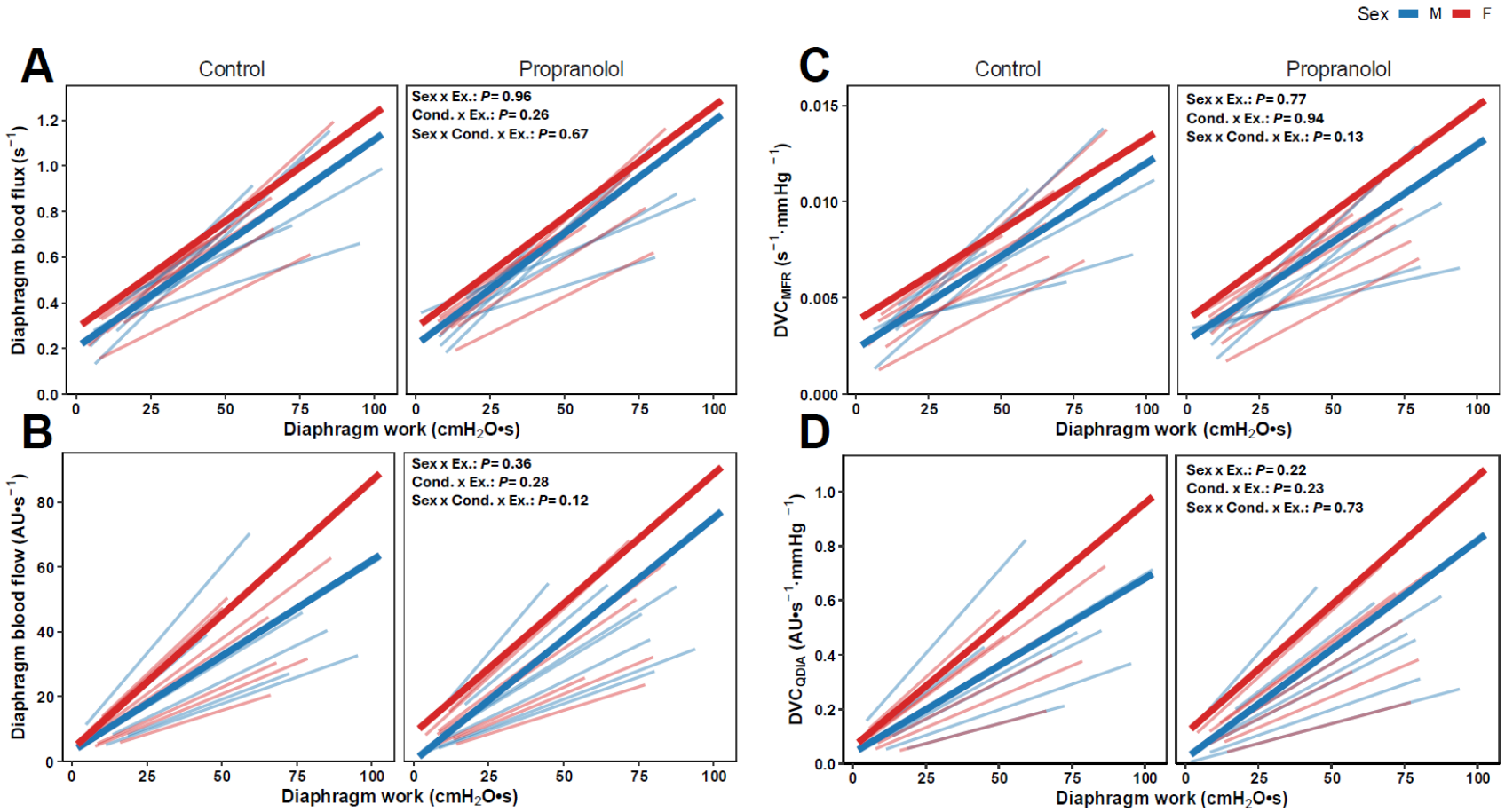


Figure 21. Mean linear regression model of diaphragm hyperemia responses over diaphragm work

Mean linear regression of diaphragm blood flux (**A**), diaphragm blood flow (**B**), diaphragm vascular conductance derived from microvascular blood flow (DVC_{MFR}) (**C**), and diaphragm vascular conductance derived from diaphragm blood flow (DVC_{QDIA}) (**D**) over diaphragm work in males (blue) and females (red) during control and propranolol conditions. Bolded lines represent the mean linear regression curves.

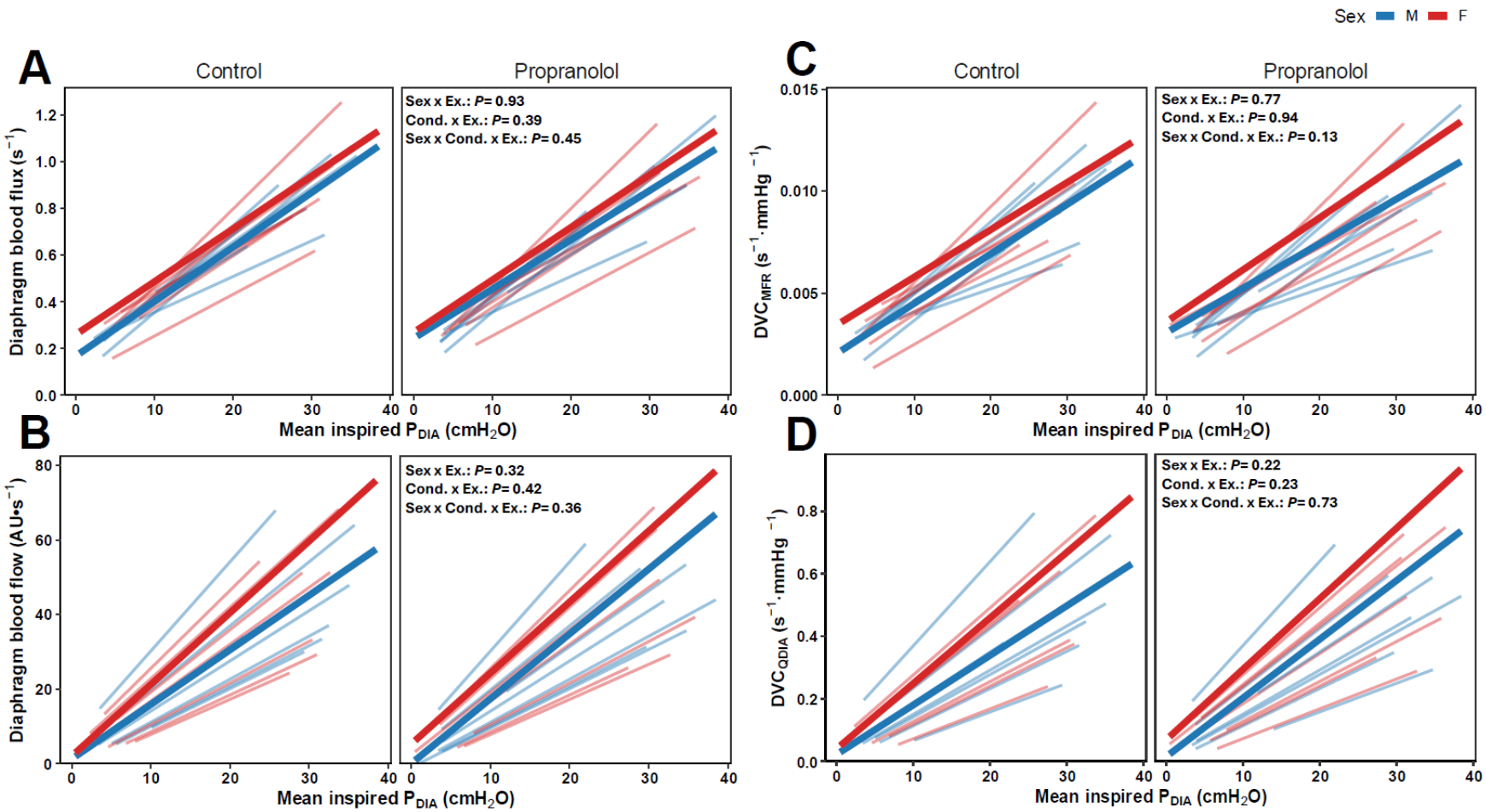


Figure 22. Mean linear regression model of the diaphragm hyperemia response over mean inspired transdiaphragmatic pressures

Mean linear regression of diaphragm blood flux (A), diaphragm blood flow (B), diaphragm vascular conductance derived from microvascular blood flow (DVC_{MFR}) (C), and diaphragm vascular conductance derived from diaphragm blood flow ($DVC_{\dot{Q}DIA}$) over mean inspired transdiaphragmatic pressure (P_{DIA}) in males (blue) and females (red) during control and propranolol conditions. Bolded lines represent the mean linear regression curves.

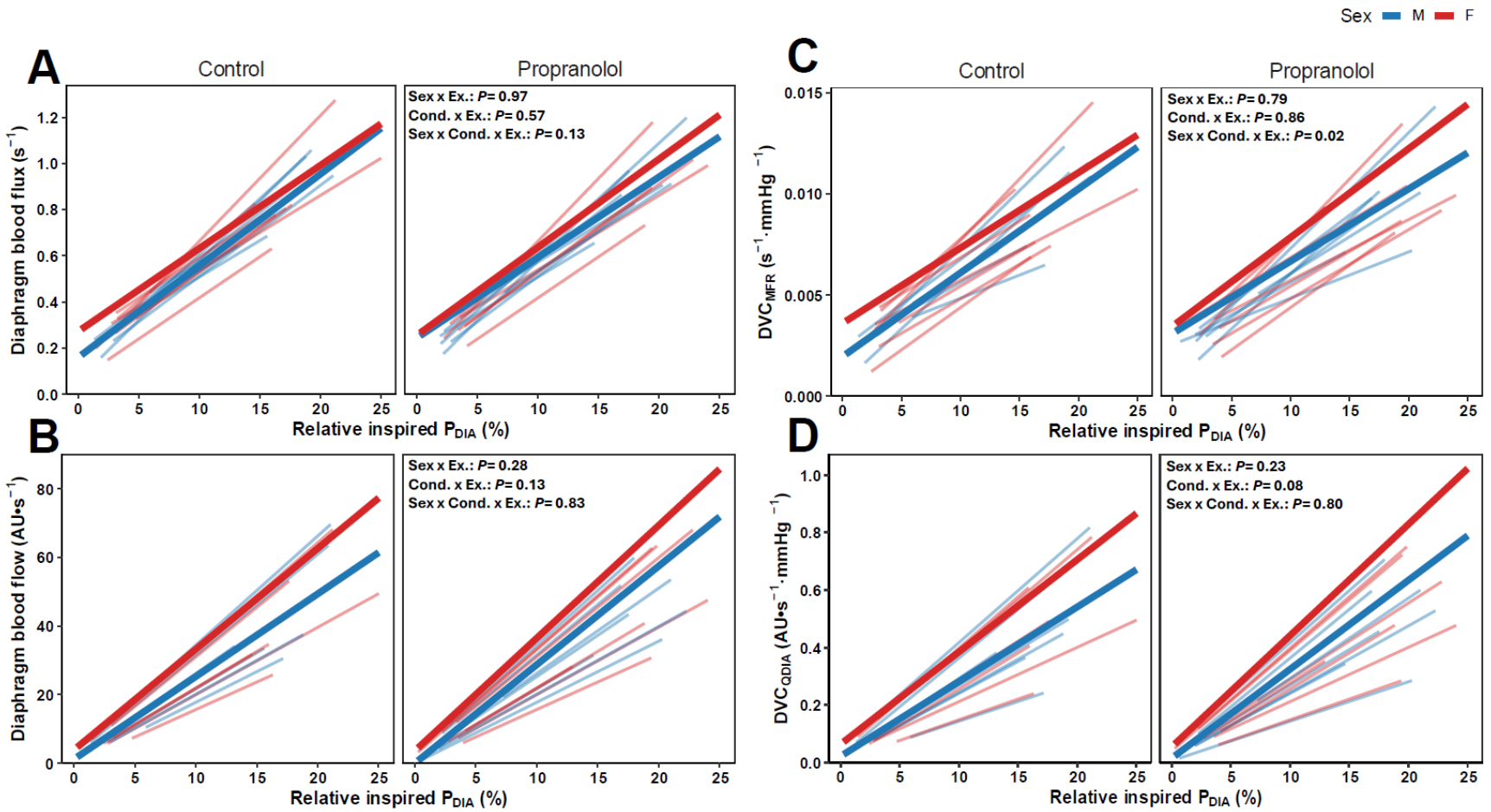


Figure 23. Mean linear regression of diaphragm hypermia responses over inspired transdiaphragmatic pressure relative to maximal transdiaphramtic pressure

Mean linear regression of diaphragm blood flux (**A**), diaphragm blood flow (**B**), diaphragm vascular conductance derived from microvascular blood flow (DVC_{MFR}) (**C**), and diaphragm vascular conductance derived from diaphragm blood flow ($DVC_{\dot{Q}DIA}$) (**D**) over inspired transdiaphragmatic pressure (P_{DIA}) relative to maximal transdiaphragmatic pressure in males (blue) and females (red) during control and propranolol conditions. Bolded lines represent the mean linear regression curves.

5.4 Discussion

We sought to determine the role of β-ARs in mediating diaphragm exercise hyperemia in males and females during incremental inspiratory threshold loading exercise. The major findings from the present study are twofold. First, there were no sex differences in the diaphragm microvascular blood flow or DVC to incremental threshold loading exercise (Figure 18). Mixed linear regression model analysis also reported sex to have no impact on the slope relationship between MFR, $\dot{Q}_{DIA}$, DVC_{MFR} and $DVC_{\dot{Q}DIA}$ versus diaphragm work, P_{DIA} and P_{DIA} relative to PDI_{MAX}. We interpret these findings to suggest males and females possess a similar ability to perfuse the active diaphragm during submaximal inspiratory loading exercise. Second, complete β-AR blockade with propranolol did not alter the diaphragm hyperemia response in either males or females. Despite reductions in resting heart rate and MAP with propranolol, increases in diaphragm perfusion and DVC during inspiratory loading were preserved. Overall, our findings suggest that β-AR mediated vasodilation is not a principal determinant of diaphragm exercise hyperemia in healthy young adults.

Sex differences in exercise hyperemia

It remains unclear if there are sex differences in skeletal muscle blood flow regulation during isolated skeletal muscle dynamic exercise. Previous work supporting sex differences in exercise hyperemia was observed during rhythmic handgrip (183, 185) or knee extension exercise (10, 11). However, present findings of no sex differences in the exercise hyperemia response to incremental diaphragm work aligns with other work examining leg (271) and arm blood flow and vascular conductance response to rhythmic exercise (252, 260). Recent work employing contrast enhanced ultrasound imaging of the diaphragm also found no significant sex difference in the slope response of $\dot{Q}_{DIA}$ over diaphragm work (137). Indeed, during a fatiguing inspiratory resistive

breathing task, MBV, MFR, $\dot{Q}_{DIA}$ and $DVC_{\dot{Q}DIA}$ was not found to be different between sexes across baseline and exercise conditions (137).

No single mechanism has been identified that could explain the variability in outcomes across the literature examining sex differences in exercise hyperemia. However, the impact of strength, size and lean body mass, and hemoglobin concentrations could provide potential explanation for sex differences in the exercise hyperemia. First, males tend to be stronger than females and achieve greater peak workloads. Strength of a contraction directly relates to the perfusion of active skeletal muscle tissue, greater muscle strength can act as a barrier to blood flow as muscular contractions increases the mechanical compression of the vasculature, increasing the resistance to blood flow and the metaboreflex blood pressure response during exercise (69, 292). Indeed, when examining the impact of handgrip strength others found that strength, not sex was a primary determinant of the exercise pressor response (69, 293). Indeed, a decrease in oxygen delivery will increase the recruitment of type II muscle fibres, which are associated with an increased accumulation of exercise metabolites (294, 295). In turn, individuals with reduced oxygen delivery and a greater build up of metabolites will experience greater muscular fatigue (295). Since no sex differences in diaphragm thickness or strength and TTI_{DIA} was well below the 0.3 threshold (Tables 3, 4) to cause a reduction in diaphragm perfusion (290), it is unsurprising that we did not observe any sex difference in $\dot{Q}_{DIA}$.

Second, males tend to have larger limb volumes and thus necessitate greater absolute blood flows at baseline and for a given exercise workload (296). Thus, when blood flows are normalized to lean body mass, previously reported sex differences have been eliminated (174, 271). Prior work by our group during single-leg knee extension found absolute leg blood flow was greater in males, however, after normalizing to estimated quadriceps muscle mass, the sex difference was

eliminated (271). Similarly, sex differences in cardiac output and leg blood flow were abolished after adjusting for lean body mass during incremental cycling exercise in highly trained cyclists (174).

Third, females tend to have lower hemoglobin blood concentrations compared to their male counterparts (297). Indeed, since arterial oxygen content is proportional to blood hemoglobin concentrations (233), greater hemoglobin concentrations in males leads higher oxygen delivery for a given volume of blood, even after normalizing to lean body mass (174). Other studies examining hyperemia responses during hypoxia similarly demonstrates how blood flow is increased to exercising tissue relative to control conditions due to reductions in blood oxygen content (233). Present study did not measure blood hemoglobin concentrations nor estimate diaphragm lean mass and since CEUS determination of $\dot{Q}_{DIA}$ is only a relative measure of microvascular tissue perfusion, results cannot be adjusted for potential sex differences in diaphragm morphology or blood hemoglobin concentrations.

Beta-adrenergic receptors and exercise hyperemia

Previous work suggested a role for an estrogen-related mechanism in mediating sex differences in exercise hyperemia when they reported young males and older post-menopausal had a blunted leg blood flow and vascular conductance response compared to young females (11). Increased estrogen is understood to play a role in upregulating the distribution and sensitivity of β-AR throughout the cardiovascular system (298). Indeed, females have been shown to have greater β-AR vasodilator responsiveness and a reduced α-adrenergic vasoconstrictor response at rest (8, 9). When females β-AR receptors were blocked with propranolol, the α-adrenergic vasoconstrictor response increased to levels observed in males (8). Present findings of no sex differences in the diaphragm hyperemic response with or without propranolol adds to the evidence

refuting the notion that β-AR are involved in mediating the exercise hyperemia response. Indeed, other studies examining blood flow to the active leg or arm did not observe a change in the overall hyperemic response in males or females to incremental exercise with β-AR blockade (233, 254, 271, 273). Interestingly, a significant sex × condition interaction was observed for the relationship between DVC_{MFR} and inspired P_{DIA} relative to PDI_{MAX} (Figure 23C). However, this interaction could be explained by the greater relative drop in MAP during the propranolol condition compared to control experienced by female participants due to their lower absolute MAP, and greater determination of DVC.

Our current results further support the idea of multiple compensatory pathways activating to preserve the hyperemia response during exercise (1). Other studies which blocked a single vasodilatory mechanism have found no differences in the exercise hyperemia response (67, 81, 247, 299). Only when multiple vasodilating pathways were inhibited was the exercise hyperemia response significantly blunted (67, 247, 249, 299). Thus, the presence of multiple vasodilating mechanisms highlights the priority which is placed in preservice oxygen delivery to high demand exercising tissues (1, 77, 248).

Perspectives on the diaphragm hyperemia response in males and females

Sex differences in the morphology of the lungs have been well documented. Females tend to have smaller lung sizes, airways and have a smaller more prismatic rib cage (14, 197, 203). Functionally, this results in a greater work of breathing and oxygen cost of breathing for females (157, 211). However, given the low-intensity inspiratory threshold loading exercise protocol in the present study, we hypothesized male and female diaphragm hyperemia response would not be different. In line with our hypothesis, males and females demonstrated similar increases in $\dot{Q}_{DIA}$, MFR, and DVC with increases in inspiratory pressure and diaphragm work. Although females

demonstrated greater resting $\dot{Q}_{DIA}$ and $DVC_{\dot{Q}DIA}$, these baseline differences can be explained by the non-significantly larger MBV and lower absolute MAP reported in females. Nonetheless, measures of $\dot{Q}_{DIA}$ and $DVC_{\dot{Q}DIA}$ did not differ during exercise and did not translate to a difference in the overall exercise hyperemia responses. Additionally, present study measures of $\dot{Q}_{DIA}$ with CEUS are limited as they are a relative measure of microvascular perfusion rather than an absolute measure of blood flow. This limitation can be attributable several factors, including only imaging of the right coastal diaphragm was performed, potential variability in the activation and concentration of the contrast agent, and the standardized dose of the contrast agent, despite interindividual differences in body mass.

Methodological Considerations

There are several methodological considerations that warrant discussion. Contrast enhanced ultrasound measures of diaphragm perfusion were taken during an end-expiration apnea, as it is not possible to acquire continuous imaging of the diaphragm during respiratory maneuvers. Thus, the blood flow data is representative of the post-exercise hyperemic response rather than a continuous blood flow measure. Over the course of breathing during an incremental ITL challenge, participants experienced an increase in inspiratory capacity (Table 11) and dropped further into their expiratory reserve volume increasing the stretch of diaphragm muscle fibres (137, 300). Thus, the increased fibre length may result in greater attenuations of $\dot{Q}_{DIA}$ and CEUS measures of diaphragm perfusion underestimated.

CEUS measures of Q_{DIA} are dependent on contrast concentrations, several factors may confound the ability to measures MBV. First, participants received the same volume and concentration of the contrast agent. Therefore, smaller individuals with smaller blood volumes will have a greater concentration of circulating contrast bubbles, which can lead to a greater MBV

value and possible over-estimations of $\dot{Q}_{DIA}$. Second, it is challenging to identify the number of microvessels in the ROI containing microbubbles as the low microbubble to red blood cell ratio and an irregular distribution of red blood cells in the microvasculature may result in the microvasculature not receiving a single microbubble (301, 302). Third, as exercise intensity increases, blood flow and microvascular hematocrit will increase, potentially reducing the number of microbubbles for a given blood flow, further confounding interpretation of MBV (303). Given these discrepancies, the present study places a greater emphasis on MFR as the primary indicator of diaphragm perfusion, rather than $\dot{Q}_{DIA}$ and MBV.

5.5 Conclusions

We investigated the role of β-ARs in mediating possible sex differences in diaphragm exercise hyperemia during inspiratory loading exercise. In agreement with our hypothesis, we found no sex differences in the diaphragm hyperemia response and no evidence that β-ARs significantly contribute to increases in diaphragm blood flow or vascular conductance during inspiratory exercise. These findings add to the literature outlining the predominant role of local metabolic vasodilatory mechanisms in regulating skeletal muscle perfusion during exercise and further emphasizes the redundant nature of mechanisms responsible for maintaining oxygen delivery to active skeletal muscle.

Chapter 6: Extended discussion

6.1 Overview

Exercise hyperemia is a critical process to ensure adequate oxygen is supplied to active skeletal muscle tissues. Whether sex impacts the exercise hyperemia response remain unknown (10, 11, 183, 185, 252, 260, 271, 273). Sex differences in β-AR activity have been identified as a mechanism which account for sex differences in the regulation of resting blood pressures and α-AR mediated vasoconstrict in young males and females (8, 9). However, whether differences in β-AR activity account for previously reported sex differences in exercise hyperemia have not yet been investigated. This thesis sought to directly investigate the presence of sex differences in exercise hyperemia and whether they were regulated by β-AR activity. Thus, three separate studies were conducted to examine the exercise hyperemia response in males and females during isolated locomotor muscle (Chapters 3), small muscle mass (Chapter 4) and the diaphragm (Chapter 5) exercise with and without systemic β-AR blockade.

6.2 Summary of findings

In Chapter 3, incremental knee extension exercise to fatigue was performed in males and females with and without intravenous infusion of propranolol. Although absolute $\dot{Q}_{LEG}$ and LVC differed between sexes, the slope of the relationship between $\dot{Q}_{LEG}$ and LVC over incremental work was similar between sexes and remained unchanged after normalizing for quadriceps muscle mass. Interestingly, females were found to have greater femoral artery dilation during exercise, which was more significantly blunted by propranolol than males. These findings suggest that β-AR do not play a major role in regulating exercise hyperemia but may contribute to sex differences in conduit artery vascular tone during exercise.

Previous work in hypoxia suggested that the input of β-AR in mediating the hyperemia response is only observed during low-intensity handgrip exercise (272). Therefore, to test whether β-AR only mediated the hyperemia during low-exercise intensity, small muscle mass exercise, Chapter 4 investigated the exercise hyperemia response to the arm during incremental and low intensity rhythmic handgrip exercise. Despite greater absolute $\dot{Q}_{ARM}$ and VC_{ARM} responses in males, the overall hyperemia response was similar between sexes and remained unchanged during β-AR blockade. In addition, refuting our hypothesis, changes in $\dot{Q}_{ARM}$ and VC_{ARM} were not different during low-intensity exercise with β-AR blockade. Consistent with our findings from Chapter 3, females demonstrated a greater propranolol-induced blunting of brachial artery dilation than males, further supporting a sex-specific role for β-AR in the regulation of conduit artery tone.

In the final study, the role of β-AR in mediating potential sex differences in diaphragm hyperemia was examined during incremental inspiratory threshold loading exercise. Diaphragm microvascular blood flow and vascular conductance responses were similar between males and females and were unaffected by β-AR blockade. Thus, like locomotor skeletal muscle, diaphragm hyperemia appears to be regulated similarly between sexes, independent of sex differences in β-ARs.

Collectively, these findings demonstrate that the exercise hyperemia response is preserved across sexes during systemic β-AR blockade in large and small locomotor muscles and the diaphragm. Although β-AR activity does not appear to play a critical role in mediating exercise hyperemia, the greater propranolol induced attenuation of conduit artery dilation in females suggests a sex-specific role for β-ARs in regulating macrovascular tone during exercise.

6.3 Significance and perspectives

Sex differences in exercise hyperemia

This thesis performed three studies directly measuring the hyperemia response to the arm, leg and diaphragm. Although males exhibited greater absolute $\dot{Q}_{LEG}$ and $\dot{Q}_{ARM}$ values, the overall hyperemia response, assessed by the slope of blood flow and vascular conductance across incremental exercise intensities, was not different between sexes. Given the findings of this thesis do not support β-ARs as a mechanism mediating sex differences in exercise hyperemia, there remains no clear explanation for the discrepancies reported in the literature. However, sex differences in hemoglobin concentrations, and anthropometrics could account for this variability.

Young healthy females generally possess lower blood hemoglobin concentrations than males (304). Because hemoglobin concentration greatly influences arterial oxygen content, individuals with lower hemoglobin concentrations would require greater blood flows to deliver the same amount of oxygen. In Chapter 3, blood hemoglobin concentrations were measured, but no sex differences in the estimated O_2 delivery was observed. In contrast, others have reported persistent sex differences in the hyperemia response following adjustment for hemoglobin concetration (10). More recently, sex differences in blood hemoglobin concentration accounted for a significant differences in estimated O_2 delivery during whole-body cycling exercise (174).

Sex differences in body size and lean body mass likely account for many of the absolute differences in blood flow reported between males and females. In Chapters 3 and 4, males were taller and larger, corresponding to larger absolute blood flow responses. However, after accounting for estimated quadriceps muscle mass, sex differences in $\dot{Q}_{LEG}$ were eliminated in Chapter 3. Indeed, accounting for lean body mass appears critical, as others similarly found no differences to the exercising limb after normalizing for lean mass (174).

Redundancy in exercise hyperemia and Beta-adrenergic receptors

The exercise hyperemia response is a critical process regulated by several redundant pathways (1). When a single vasodilator pathway is inhibited, the hyperemia response is generally preserved (79–81). Only when multiple pathways are inhibited are measurable reductions in the hyperemia response observed (67, 82, 83). Thus, numerous compensatory mechanisms exist to ensure blood flow and oxygen delivery are maintained during exercise (1). Throughout this thesis, participants were tested during β-AR blockade, yet no significant differences in the overall hyperemia response were observed in the arm, leg, or diaphragm. This preservation of the exercise hyperemia response during β-AR blockade is therefore consistent with the redundant nature of the hyperemic responses and highlights the physiological importance of maintaining oxygen delivery to active muscle during exercise.

Sex differences in conduit artery vasodilation

In Chapter 3 and 4, females exhibited greater femoral and brachial artery dilation relative to baseline during incremental fatiguing exercise. During systemic β-AR blockade, females experienced a greater blunting of conduit artery dilation than males, suggesting a sex-specific role for β-AR in the regulation of conduit artery vascular tone. Shear rate was also examined, and, unsurprisingly, larger absolute shear rate values were observed in females due to their smaller vessel diameters. However, when the slope of shear rate across absolute and relative workloads was examined, no significant differences were observed between sexes or during propranolol administration. Together, these findings suggest β-AR activity contributes to conduit artery dilation during exercise to a greater extent in females than males. Since both β-AR-mediated vasodilation and shear-mediated dilation of conduit arteries involve the nitric oxide signalling

pathway, future studies are warrened to further examine the relationship between β-AR activity, and conduit artery vasodilation during exercise (266, 267).

6.4 Considerations

Specific methodological considerations associated with individual study methodologies and study designs are discussed within each chapter. However, several broader considerations should be acknowledged when interpreting the findings of this thesis.

First, all studies included healthy young adults in sex-balanced designs specifically powered for primary outcomes. This limits the generalizability of the results to older adults, clinical populations or larger population samples. Additionally, participants recruited across all three studies were, on average, more aerobically fit than age-matched normative values (305), therefore, caution should be used when extending these findings to a more representative fitness population.

Second, this thesis employed both intravenous (Chapter 3) and oral (Chapters 4 and 5) propranolol administration to achieve systemic β-AR blockade. Intravenous propranolol has been shown to reduce HR and $\dot{Q}$, with minimal effects on resting blood pressures (8, 228, 271), a finding which was confirmed in Chapter 3. In contrast, oral propranolol administration is often associated with reductions in HR, $\dot{Q}$, and MAP (217, 238, 306). These reductions in blood pressure are generally observed following multiple doses and are attributed to the chronic effects of propranolol administration (307). To minimize these effects, Chapters 4 and 5 employed a two-dose oral propranolol protocol. Although resting MAP was reduced during propranolol administration in

Chapter 5, MAP responses to exercise were similar between conditions in both studies, suggesting that exercise-related hemodynamics were largely preserved despite differences in baseline MAP.

Third, this thesis investigated the role of β-AR in local skeletal muscle tissue using the non-selective β-AR antagonist propranolol. At present, there is no Health Canada (or other national agency) approved selective β_2-AR antagonist available for human use. Consequently, β_1-AR blockade also occurred, producing reductions in HR and $\dot{Q}$ (217). However, the use of isolated limb and diaphragm exercise models minimized the influence of reduced cardiac output on tissue perfusion and exercise performance during β-AR blockade.

Finally, methodological differences in the quantification of blood flow may contribute to discrepancies in the literature regarding sex differences in exercise hyperemia. Numerous studies assess blood flow using Doppler ultrasound imaging; however, differences in Doppler velocity processing appear to influence reported blood flow responses. Studies using Doppler audio signal processing have reported a plateau in blood flows during incremental knee extension exercise (10, 11, 101), whereas studies using intensity-weighted pixel analysis of the Doppler velocity tracing, including Chapter 3, observed a linear increase in blood flow with workload (91). Whether differences in doppler processing contributes to conflicting reports of sex differences in exercise hyperemia warrants further investigation.

6.5 Future directions

There is strong evidence for a role for β-AR in mediating sex differences in cardiovascular regulation at rest (8, 9, 308). However, β-AR are also located in the smooth muscles of airways, mediating an increase in airway calibre with elevations in sympathetic activity. Whether sex

differences in β-AR mediated regulation of airway smooth muscle has not been thoroughly investigated. In a study performed in conjunction with Chapter 3, FVC maneuvers were performed with and without propranolol before and after exercise to examine the impact of β-AR blockade on exercise induced bronchodilation (309). Despite no differences in the overall bronchodilation response as measured by FEV_1 and FEV_1/FVC between sexes, a blunting of flows at 50% and 25% FVC in males and females points to a role for β-AR in mediating airway tone in smaller airways following exercise (309). Additionally, an increase in mean slope ratio in females but not males suggest a sex difference in the distribution of β-AR in smooth muscle airways. Further studies examining the potential role of β-AR in mediating potential sex differences in airway tone at rest and following exercise are warranted.

Additionally, Given the influence of hemoglobin concentration and lean body mass on exercise hyperemia response (174, 271), these factors should be considered when evaluating sex differences in the hyperemic response. Future studies should therefore include measures of hemoglobin concentration and normalize blood flow and vascular conductance to the lean mass of the exercising limb.

6.6 Thesis conclusion

This thesis adds to the understanding of basic human physiology and the mechanisms underlying sex differences in cardiovascular regulation. Regardless of sex, a complete picture of basic human physiology and the mechanisms involved in regulating the cardiovascular system during exercise has not yet been fully illuminated. While the physiological goal of exercise remains similar between males and females (*i.e.* maintaining perfusion pressure and oxygen delivery), present findings did not identify a sex difference in the overall hyperemia response during isolated

locomotor or respiratory muscle exercise. Furthermore, systemic blockade of β-AR did not alter the exercise hyperemia response in either sex. However, the greater blunting of conduit artery dilation observed in females suggests a sex-specific role for β-AR in governing macrovascular tone during exercise. Considering the numerous proposed sex differences in cardiovascular regulation and the established role of β-AR in resting hemodynamics, continued investigation of exercise physiology in both males and females remains essential. Indeed, physiological differences observed at rest may not necessarily translate to differences in exercise responses.

References

1. **Joyner MJ**, **Casey DP**. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev* 95: 549–601, 2015. doi: 10.1152/physrev.00035.2013.

2. **Andersen P**, **Saltin B**. Maximal perfusion of skeletal muscle in man. *J Physiol* 366: 233–249, 1985.

3. **Guenette JA**, **Vogiatzis I**, **Zakynthinos S**, **Athanasopoulos D**, **Koskolou M**, **Golemati S**, **Vasilopoulou M**, **Wagner HE**, **Roussos C**, **Wagner PD**, **Boushel R**. Human respiratory muscle blood flow measured by near-infrared spectroscopy and indocyanine green. *J Appl Physiol (1985)* 104: 1202–1210, 2008. doi: 10.1152/japplphysiol.01160.2007.

4. **Writing Group Members**, **Mozaffarian D**, **Benjamin EJ**, **Go AS**, **Arnett DK**, **Blaha MJ**, **Cushman M**, **Das SR**, **de Ferranti S**, **Després J-P**, **Fullerton HJ**, **Howard VJ**, **Huffman MD**, **Isasi CR**, **Jiménez MC**, **Judd SE**, **Kissela BM**, **Lichtman JH**, **Lisabeth LD**, **Liu S**, **Mackey RH**, **Magid DJ**, **McGuire DK**, **Mohler ER**, **Moy CS**, **Muntner P**, **Mussolino ME**, **Nasir K**, **Neumar RW**, **Nichol G**, **Palaniappan L**, **Pandey DK**, **Reeves MJ**, **Rodriguez CJ**, **Rosamond W**, **Sorlie PD**, **Stein J**, **Towfighi A**, **Turan TN**, **Virani SS**, **Woo D**, **Yeh RW**, **Turner MB**, **American Heart Association Statistics Committee**, **Stroke Statistics Subcommittee**. Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation* 133: e38-360, 2016. doi: 10.1161/CIR.0000000000000350.

5. **Carter JR**, **Fu Q**, **Minson CT**, **Joyner MJ**. Ovarian cycle and sympathoexcitation in premenopausal women. *Hypertension* 61: 395–399, 2013. doi: 10.1161/HYPERTENSIONAHA.112.202598.

6. **Keir DA**, **Badrov MB**, **Tomlinson G**, **Notarius CF**, **Kimmerly DS**, **Millar PJ**, **Shoemaker JK**, **Floras JS**. Influence of Sex and Age on Muscle Sympathetic Nerve Activity of Healthy Normotensive Adults. *Hypertension* 76: 997–1005, 2020. doi: 10.1161/HYPERTENSIONAHA.120.15208.

7. **Ganzeboom KS**, **Colman N**, **Reitsma JB**, **Shen WK**, **Wieling W**. Prevalence and triggers of syncope in medical students. *American Journal of Cardiology* 91: 1006–1008, 2003. doi: 10.1016/S0002-9149(03)00127-9.

8. **Hart EC**, **Charkoudian N**, **Wallin BG**, **Curry TB**, **Eisenach J**, **Joyner MJ**. Sex and ageing differences in resting arterial pressure regulation: the role of the β-adrenergic receptors. *J Physiol* 589: 5285–5297, 2011. doi: 10.1113/jphysiol.2011.212753.

9. **Kneale BJ**, **Chowienczyk PJ**, **Brett SE**, **Coltart DJ**, **Ritter JM**. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *J Am Coll Cardiol* 36: 1233–1238, 2000.

10. **Parker BA**, **Smithmyer SL**, **Pelberg JA**, **Mishkin AD**, **Herr MD**, **Proctor DN**. Sex differences in leg vasodilation during graded knee extensor exercise in young adults. *J Appl Physiol* 103: 1583–1591, 2007. doi: 10.1152/japplphysiol.00662.2007.

11. **Parker BA**, **Smithmyer SL**, **Pelberg JA**, **Mishkin AD**, **Proctor DN**. Sex-specific influence of aging on exercising leg blood flow. *Journal of Applied Physiology* 104: 655–664, 2008. doi: 10.1152/japplphysiol.01150.2007.

12. **Dominelli PB**, **Molgat-Seon Y**. Sex, gender and the pulmonary physiology of exercise. *Eur Respir Rev* 31: 210074, 2022. doi: 10.1183/16000617.0074-2021.

13. **Dominelli PB**, **Molgat-Seon Y**, **Sheel AW**. Sex Differences in the Pulmonary System Influence the Integrative Response to Exercise. *Exercise and Sport Sciences Reviews* 47: 142–150, 2019. doi: 10.1249/JES.0000000000000188.

14. **Mann LM**, **Angus SA**, **Doherty CJ**, **Dominelli PB**. Evaluation of sex-based differences in airway size and the physiological implications. *Eur J Appl Physiol* 121: 2957–2966, 2021. doi: 10.1007/s00421-021-04778-2.

15. **Molgat-Seon Y**, **Dominelli PB**, **Guenette JA**, **Sheel AW**. Modelling the effects of age and sex on the resistive and viscoelastic components of the work of breathing during exercise. *Experimental Physiology* 104: 1737–1745, 2019. doi: 10.1113/EP087956.

16. **Guenette JA**, **Querido JS**, **Eves ND**, **Chua R**, **Sheel AW**. Sex differences in the resistive and elastic work of breathing during exercise in endurance-trained athletes. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 297: R166–R175, 2009. doi: 10.1152/ajpregu.00078.2009.

17. **Stickland MK**, **Lindinger MI**, **Olfert IM**, **Heigenhauser GJF**, **Hopkins SR**. Pulmonary gas exchange and acid-base balance during exercise. *Compr Physiol* 3: 693–739, 2013. doi: 10.1002/cphy.c110048.

18. **Hughson RL**, **Tschakovsky ME**, **Houston ME**. Regulation of oxygen consumption at the onset of exercise. *Exerc Sport Sci Rev* 29: 129–133, 2001. doi: 10.1097/00003677-200107000-00008.

19. **West JB**. *Respiratory Physiology: The Essentials*. Lippincott Williams & Wilkins, 2012.

20. **Nobrega ACL**, **O'Leary D**, **Silva BM**, **Marongiu E**, **Piepoli MF**, **Crisafulli A**. Neural Regulation of Cardiovascular Response to Exercise: Role of Central Command and Peripheral Afferents. *Biomed Res Int* 2014: 478965, 2014. doi: 10.1155/2014/478965.

21. **Williamson JW**. The relevance of central command for the neural cardiovascular control of exercise. *Exp Physiol* 95: 1043–1048, 2010. doi: 10.1113/expphysiol.2009.051870.

22. **Victor RG**, **Secher NH**, **Lyson T**, **Mitchell JH**. Central command increases muscle sympathetic nerve activity during intense intermittent isometric exercise in humans. *Circ Res* 76: 127–131, 1995.

23. **Sagawa K**. Baroreflex Control of Systemic Arterial Pressure and Vascular Bed. In: *Comprehensive Physiology*. American Cancer Society, p. 453–496.

24. **Ray CA**, **Rea RF**, **Clary MP**, **Mark AL**. Muscle sympathetic nerve responses to dynamic one-legged exercise: effect of body posture. *Am J Physiol Heart Circ Physiol* 264: H1–H7, 1993. doi: 10.1152/ajpheart.1993.264.1.H1.

25. **Mitchell JH**, **Kaufman MP**, **Iwamoto GA**. The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. *Annu Rev Physiol* 45: 229–242, 1983. doi: 10.1146/annurev.ph.45.030183.001305.

26. **Cui J**, **Blaha C**, **Moradkhan R**, **Gray KS**, **Sinoway LI**. Muscle sympathetic nerve activity responses to dynamic passive muscle stretch in humans. *J Physiol* 576: 625–634, 2006. doi: 10.1113/jphysiol.2006.116640.

27. **Keir DA**, **Duffin J**, **Millar PJ**, **Floras JS**. Simultaneous assessment of central and peripheral chemoreflex regulation of muscle sympathetic nerve activity and ventilation in healthy young men. *The Journal of Physiology* 597: 3281–3296, 2019. doi: https://doi.org/10.1113/JP277691.

28. **Dampney RA**. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 74: 323–364, 1994. doi: 10.1152/physrev.1994.74.2.323.

29. **Goodwin GM**, **McCloskey DI**, **Mitchell JH**. Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. *J Physiol* 226: 173–190, 1972.

30. **Stebbins CL**, **Brown B**, **Levin D**, **Longhurst JC**. Reflex effect of skeletal muscle mechanoreceptor stimulation on the cardiovascular system. *J Appl Physiol* 65: 1539–1547, 1988. doi: 10.1152/jappl.1988.65.4.1539.

31. **Greaney JL**, **Wenner MM**, **Farquhar WB**. Exaggerated increases in blood pressure during isometric muscle contraction in hypertension: Role for purinergic receptors. *Autonomic Neuroscience* 188: 51–57, 2015. doi: 10.1016/j.autneu.2014.12.003.

32. **Incognito AV**, **Doherty CJ**, **Lee JB**, **Burns MJ**, **Millar PJ**. Ischemic preconditioning does not alter muscle sympathetic responses to static handgrip and metaboreflex activation in young healthy men. *Physiol Rep* 5, 2017. doi: 10.14814/phy2.13342.

33. **Mark AL**, **Victor RG**, **Nerhed C**, **Wallin BG**. Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circ Res* 57: 461–469, 1985.

34. **Kaufman MP**, **Longhurst JC**, **Rybicki KJ**, **Wallach JH**, **Mitchell JH**. Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *J Appl Physiol Respir Environ Exerc Physiol* 55: 105–112, 1983. doi: 10.1152/jappl.1983.55.1.105.

35. **Potts JT**, **Shi XR**, **Raven PB**. Carotid baroreflex responsiveness during dynamic exercise in humans. *Am J Physiol Heart Circ Physiol* 265: H1928–H1938, 1993. doi: 10.1152/ajpheart.1993.265.6.H1928.

36. **Fadel PJ**, **Ogoh S**, **Keller DM**, **Raven PB**. Recent insights into carotid baroreflex function in humans using the variable pressure neck chamber. *Exp Physiol* 88: 671–680, 2003.

37. **Fisher JP**, **Young CN**, **Fadel PJ**. Effect of muscle metaboreflex activation on carotid-cardiac baroreflex function in humans. *Am J Physiol Heart Circ Physiol* 294: H2296-2304, 2008. doi: 10.1152/ajpheart.91497.2007.

38. **McIlveen SA**, **Hayes SG**, **Kaufman MP**. Both central command and exercise pressor reflex reset carotid sinus baroreflex. *Am J Physiol Heart Circ Physiol* 280: H1454-1463, 2001. doi: 10.1152/ajpheart.2001.280.4.H1454.

39. **Doherty CJ**, **Incognito AV**, **Notay K**, **Burns MJ**, **Slysz JT**, **Seed JD**, **Nardone M**, **Burr JF**, **Millar PJ**. Muscle sympathetic nerve responses to passive and active one-legged cycling: insights into the contributions of central command. *Am J Physiol Heart Circ Physiol* 314: H3–H10, 2018. doi: 10.1152/ajpheart.00494.2017.

40. **Krogh A**, **Lindhard J**. The regulation of respiration and circulation during the initial stages of muscular work. *J Physiol* 47: 112–136, 1913.

41. **Thornton JM**, **Pederson DL**, **Kardos A**, **Guz A**, **Casadei B**, **Paterson DJ**. Ventilatory response to imagination of exercise and altered perception of exercise load under hypnosis. *Adv Exp Med Biol* 450: 195–197, 1998. doi: 10.1007/978-1-4757-9077-1_31.

42. **Miyamoto T**, **Sotobayashi D**, **Ito G**, **Kawai E**, **Nakahara H**, **Ueda S**, **Toyama T**, **Saku K**, **Nakanishi Y**, **Kinoshita H**. Physiological role of anticipatory cardiorespiratory responses to exercise. *Physiol Rep* 10: e15210, 2022. doi: 10.14814/phy2.15210.

43. **Amann M**, **Venturelli M**, **Ives SJ**, **Morgan DE**, **Gmelch B**, **Witman MAH**, **Jonathan Groot H**, **Walter Wray D**, **Stehlik J**, **Richardson RS**. Group III/IV muscle afferents impair limb blood in patients with chronic heart failure. *Int J Cardiol* 174: 368–375, 2014. doi: 10.1016/j.ijcard.2014.04.157.

44. **Van Brummelen P**, **Jie K**, **Van Zwieten P**. Alpha-adrenergic receptors in human blood vessels. *British Journal of Clinical Pharmacology* 21: 33S-39S, 1986. doi: 10.1111/j.1365-2125.1986.tb02851.x.

45. **Barnes PJ**. β-adrenoceptors on smooth muscle, nerves and inflammatory cells. *Life Sciences* 52: 2101–2109, 1993. doi: 10.1016/0024-3205(93)90725-I.

46. **Buckwalter JB**, **Clifford PS**. alpha-adrenergic vasoconstriction in active skeletal muscles during dynamic exercise. *Am J Physiol* 277: H33-39, 1999. doi: 10.1152/ajpheart.1999.277.1.H33.

47. **Mora-Rodriguez R**, **Coyle EF**. Effects of plasma epinephrine on fat metabolism during exercise: interactions with exercise intensity. *American Journal of Physiology-Endocrinology and Metabolism* 278: E669–E676, 2000. doi: 10.1152/ajpendo.2000.278.4.E669.

48. **Saito M**, **Tsukanaka A**, **Yanagihara D**, **Mano T**. Muscle sympathetic nerve responses to graded leg cycling. *Journal of Applied Physiology* 75: 663–667, 1993. doi: 10.1152/jappl.1993.75.2.663.

49. **Marshall RJ**, **Schirger A**, **Shepherd JT**. Blood pressure during supine exercise in idiopathic orthostatic hypotension. *Circulation* 24: 76–81, 1961.

50. **Secher NH**, **Clausen JP**, **Klausen K**, **Noer I**, **Trap-Jensen J**. Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiol Scand* 100: 288–297, 1977. doi: 10.1111/j.1748-1716.1977.tb05952.x.

51. **Volianitis S**, **Secher NH**. Arm blood flow and metabolism during arm and combined arm and leg exercise in humans. *J Physiol* 544: 977–984, 2002. doi: 10.1113/jphysiol.2002.023556.

52. **Harms CA**, **Babcock MA**, **McClaran SR**, **Pegelow DF**, **Nickele GA**, **Nelson WB**, **Dempsey JA**. Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol (1985)* 82: 1573–1583, 1997. doi: 10.1152/jappl.1997.82.5.1573.

53. **Harms CA**, **Wetter TJ**, **St Croix CM**, **Pegelow DF**, **Dempsey JA**. Effects of respiratory muscle work on exercise performance. *J Appl Physiol (1985)* 89: 131–138, 2000. doi: 10.1152/jappl.2000.89.1.131.

54. **Remensnyder JP**, **Mitchell JH**, **Sarnoff SJ**. Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res* 11: 370–380, 1962.

55. **Rosenmeier JB**, **Dinenno FA**, **Fritzlar SJ**, **Joyner MJ**. alpha1- and alpha2-adrenergic vasoconstriction is blunted in contracting human muscle. *J Physiol* 547: 971–976, 2003. doi: 10.1113/jphysiol.2002.037937.

56. **Tschakovsky ME**, **Sujirattanawimol K**, **Ruble SB**, **Valic Z**, **Joyner MJ**. Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? *J Physiol* 541: 623–635, 2002. doi: 10.1113/jphysiol.2001.014431.

57. **Faber JE**, **Meininger GA**. Selective interaction of alpha-adrenoceptors with myogenic regulation of microvascular smooth muscle. *Am J Physiol* 259: H1126-1133, 1990. doi: 10.1152/ajpheart.1990.259.4.H1126.

58. **Anderson KM**, **Faber JE**. Differential sensitivity of arteriolar alpha 1- and alpha 2-adrenoceptor constriction to metabolic inhibition during rat skeletal muscle contraction. *Circ Res* 69: 174–184, 1991.

59. **Ohyanagi M**, **Nishigaki K**, **Faber JE**. Interaction between microvascular alpha 1- and alpha 2-adrenoceptors and endothelium-derived relaxing factor. *Circ Res* 71: 188–200, 1992. doi: 10.1161/01.res.71.1.188.

60. **Tateishi J**, **Faber JE**. Inhibition of arteriole alpha 2- but not alpha 1-adrenoceptor constriction by acidosis and hypoxia in vitro. *Am J Physiol* 268: H2068-2076, 1995. doi: 10.1152/ajpheart.1995.268.5.H2068.

61. **McGillivray-Anderson KM**, **Faber JE**. Effect of acidosis on contraction of microvascular smooth muscle by alpha 1- and alpha 2-adrenoceptors. Implications for neural and metabolic regulation. *Circ Res* 66: 1643–1657, 1990. doi: 10.1161/01.res.66.6.1643.

62. **Hughson RL**. Regulation of Blood Flow at the Onset of Exercise by Feed Forward and Feedback Mechanisms. .

63. **Hník P**, **Holas M**, **Krekule I**, **Kŭriz N**, **Mejsnar J**, **Smiesko V**, **Ujec E**, **Vyskocil F**. Work-induced potassium changes in skeletal muscle and effluent venous blood assessed by liquid ion-exchanger microelectrodes. *Pflugers Arch* 362: 85–94, 1976. doi: 10.1007/BF00588685.

64. **Green S**, **Langberg H**, **Skovgaard D**, **Bulow J**, **Kjaer M**. Interstitial and arterial-venous [K+] in human calf muscle during dynamic exercise: effect of ischaemia and relation to muscle pain. *J Physiol* 529 Pt 3: 849–861, 2000. doi: 10.1111/j.1469-7793.2000.00849.x.

65. **Hamann JJ**, **Buckwalter JB**, **Clifford PS**. Vasodilatation is obligatory for contraction-induced hyperaemia in canine skeletal muscle. *J Physiol* 557: 1013–1020, 2004. doi: 10.1113/jphysiol.2004.062836.

66. **Casey DP**, **Walker BG**, **Ranadive SM**, **Taylor JL**, **Joyner MJ**. Contribution of nitric oxide in the contraction-induced rapid vasodilation in young and older adults. *J Appl Physiol (1985)* 115: 446–455, 2013. doi: 10.1152/japplphysiol.00446.2013.

67. **Crecelius AR**, **Kirby BS**, **Luckasen GJ**, **Larson DG**, **Dinenno FA**. Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 305: H29–H40, 2013. doi: 10.1152/ajpheart.00298.2013.

68. **Laughlin MH**. Skeletal muscle blood flow capacity: role of muscle pump in exercise hyperemia. *Am J Physiol* 253: H993-1004, 1987. doi: 10.1152/ajpheart.1987.253.5.H993.

69. **Lee JB**, **Notay K**, **Seed JD**, **Nardone M**, **Omazic LJ**, **Millar PJ**. Sex Differences in Muscle Metaboreflex Activation after Static Handgrip Exercise. *Medicine & Science in Sports & Exercise* 53: 2596, 2021. doi: 10.1249/MSS.0000000000002747.

70. **Hellsten Y**, **Maclean D**, **Rådegran G**, **Saltin B**, **Bangsbo J**. Adenosine concentrations in the interstitium of resting and contracting human skeletal muscle. *Circulation* 98: 6–8, 1998.

71. **Martin EA**, **Nicholson WT**, **Eisenach JH**, **Charkoudian N**, **Joyner MJ**. Bimodal distribution of vasodilator responsiveness to adenosine due to difference in nitric oxide contribution: implications for exercise hyperemia. *J Appl Physiol (1985)* 101: 492–499, 2006. doi: 10.1152/japplphysiol.00684.2005.

72. **González-Alonso J**, **Mortensen SP**, **Jeppesen TD**, **Ali L**, **Barker H**, **Damsgaard R**, **Secher NH**, **Dawson EA**, **Dufour SP**. Haemodynamic responses to exercise, ATP infusion and thigh compression in humans: insight into the role of muscle mechanisms on cardiovascular function. *J Physiol* 586: 2405–2417, 2008. doi: 10.1113/jphysiol.2008.152058.

73. **Mortensen SP**, **González-Alonso J**, **Bune LT**, **Saltin B**, **Pilegaard H**, **Hellsten Y**. ATP-induced vasodilation and purinergic receptors in the human leg: roles of nitric oxide, prostaglandins, and adenosine. *Am J Physiol Regul Integr Comp Physiol* 296: R1140-1148, 2009. doi: 10.1152/ajpregu.90822.2008.

74. **Rosenmeier JB**, **Hansen J**, **González-Alonso J**. Circulating ATP-induced vasodilatation overrides sympathetic vasoconstrictor activity in human skeletal muscle. *J Physiol* 558: 351–365, 2004. doi: 10.1113/jphysiol.2004.063107.

75. **Furchgott RF**. The discovery of endothelium-derived relaxing factor and its importance in the identification of nitric oxide. *JAMA* 276: 1186–1188, 1996.

76. **Furchgott RF**, **Zawadzki JV**. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373–376, 1980.

77. **Hellsten Y**, **Nyberg M**, **Jensen LG**, **Mortensen SP**. Vasodilator interactions in skeletal muscle blood flow regulation. *J Physiol* 590: 6297–6305, 2012. doi: 10.1113/jphysiol.2012.240762.

78. **Vanhoutte PM**. Endothelial adrenoceptors. *J Cardiovasc Pharmacol* 38: 796–808, 2001.

79. **Morganroth ML**, **Mohrman DE**, **Sparks HV**. Prolonged vasodilation following fatiguing exercise of dog skeletal muscle. *Am J Physiol* 229: 38–43, 1975. doi: 10.1152/ajplegacy.1975.229.1.38.

80. **Schrage WG**, **Wilkins BW**, **Johnson CP**, **Eisenach JH**, **Limberg JK**, **Dietz NM**, **Curry TB**, **Joyner MJ**. Roles of nitric oxide synthase and cyclooxygenase in leg vasodilation and oxygen consumption during prolonged low-intensity exercise in untrained humans. *J Appl Physiol (1985)* 109: 768–777, 2010. doi: 10.1152/japplphysiol.00326.2010.

81. **Shoemaker JK**, **Naylor HL**, **Pozeg ZI**, **Hughson RL**. Failure of prostaglandins to modulate the time course of blood flow during dynamic forearm exercise in humans. *J Appl Physiol (1985)* 81: 1516–1521, 1996. doi: 10.1152/jappl.1996.81.4.1516.

82. **Mortensen SP**, **González-Alonso J**, **Damsgaard R**, **Saltin B**, **Hellsten Y**. Inhibition of nitric oxide and prostaglandins, but not endothelial-derived hyperpolarizing factors, reduces

blood flow and aerobic energy turnover in the exercising human leg. *J Physiol* 581: 853–861, 2007. doi: 10.1113/jphysiol.2006.127423.

83. **Boushel R**, **Langberg H**, **Gemmer C**, **Olesen J**, **Crameri R**, **Scheede C**, **Sander M**, **Kjær M**. Combined inhibition of nitric oxide and prostaglandins reduces human skeletal muscle blood flow during exercise. *J Physiol* 543: 691–698, 2002. doi: 10.1113/jphysiol.2002.021477.

84. **Frandsen U**, **Bangsbo J**, **Langberg H**, **Saltin B**, **Hellsten Y**. Inhibition of nitric oxide synthesis by systemic N(G)-monomethyl-L-arginine administration in humans: effects on interstitial adenosine, prostacyclin and potassium concentrations in resting and contracting skeletal muscle. *J Vasc Res* 37: 297–302, 2000. doi: 10.1159/000025743.

85. **Bolz SS**, **Pohl U**. Indomethacin enhances endothelial NO release--evidence for a role of PGI2 in the autocrine control of calcium-dependent autacoid production. *Cardiovasc Res* 36: 437–444, 1997. doi: 10.1016/s0008-6363(97)00197-1.

86. **Stringer W**, **Wasserman K**, **Casaburi R**, **Porszasz J**, **Maehara K**, **French W**. Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise. *Journal of Applied Physiology* 76: 1462–1467, 1994. doi: 10.1152/jappl.1994.76.4.1462.

87. **Saltin B**, **Calbet JAL**. Point: In health and in a normoxic environment, V̇o2 max is limited primarily by cardiac output and locomotor muscle blood flow. *Journal of Applied Physiology* 100: 744–748, 2006. doi: 10.1152/japplphysiol.01395.2005.

88. **Sheel AW**, **Boushel R**, **Dempsey JA**. Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. *Journal of Applied Physiology* 125: 820–831, 2018. doi: 10.1152/japplphysiol.00189.2018.

89. **Calbet JAL**, **Gonzalez-Alonso J**, **Helge JW**, **Søndergaard H**, **Munch-Andersen T**, **Boushel R**, **Saltin B**. Cardiac output and leg and arm blood flow during incremental exercise to exhaustion on the cycle ergometer. *Journal of Applied Physiology* 103: 969–978, 2007. doi: 10.1152/japplphysiol.01281.2006.

90. **Wray DW**, **Witman MAH**, **Ives SJ**, **McDaniel J**, **Fjeldstad AS**, **Trinity JD**, **Conklin JD**, **Supiano MA**, **Richardson RS**. Progressive handgrip exercise: evidence of nitric oxide-dependent vasodilation and blood flow regulation in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 300: H1101–H1107, 2011. doi: 10.1152/ajpheart.01115.2010.

91. **Rådegran G**. Ultrasound Doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *Journal of Applied Physiology* 83: 1383–1388, 1997. doi: 10.1152/jappl.1997.83.4.1383.

92. **Green DJ**, **Bilsborough W**, **Naylor LH**, **Reed C**, **Wright J**, **O'Driscoll G**, **Walsh JH**. Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *J Physiol* 562: 617–628, 2005. doi: 10.1113/jphysiol.2004.075929.

93. **Blomstrand E**, **Rådegran G**, **Saltin B**. Maximum rate of oxygen uptake by human skeletal muscle in relation to maximal activities of enzymes in the Krebs cycle. *J Physiol* 501 (Pt 2): 455–460, 1997. doi: 10.1111/j.1469-7793.1997.455bn.x.

94. **Rådegran G**, **Saltin B**. Muscle blood f low at onset of dynamic exercise in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 274: H314–H322, 1998. doi: 10.1152/ajpheart.1998.274.1.H314.

95. **Rowell LB**, **Saltin B**, **Kiens B**, **Christensen NJ**. Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? .

96. **Richardson RS**, **Knight DR**, **Poole DC**, **Kurdak SS**, **Hogan MC**, **Grassi B**, **Wagner PD**. Determinants of maximal exercise VO2 during single leg knee-extensor exercise in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 268: H1453–H1461, 1995. doi: 10.1152/ajpheart.1995.268.4.H1453.

97. **MacDonald MJ**, **Shoemaker JK**, **Tschakovsky ME**, **Hughson RL**. Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans. *Journal of Applied Physiology* 85: 1622–1628, 1998. doi: 10.1152/jappl.1998.85.5.1622.

98. **DeLorey DS**, **Shaw CN**, **Shoemaker JK**, **Kowalchuk JM**, **Paterson DH**. The effect of hypoxia on pulmonary O2 uptake, leg blood flow and muscle deoxygenation during single-leg knee-extension exercise. *Exp Physiol* 89: 293–302, 2004. doi: 10.1113/expphysiol.2003.026864.

99. **Hanson BE**, **Proffit M**, **Gifford JR**. Vascular function is related to blood flow during high-intensity, but not low-intensity, knee extension exercise. *Journal of Applied Physiology* 128: 698–708, 2020. doi: 10.1152/japplphysiol.00671.2019.

100. **Strange S**, **Secher NH**, **Pawelczyk JA**, **Karpakka J**, **Christensen NJ**, **Mitchell JH**, **Saltin B**. Neural control of cardiovascular responses and of ventilation during dynamic exercise in man. *J Physiol* 470: 693–704, 1993. doi: 10.1113/jphysiol.1993.sp019883.

101. **Hoelting BD**, **Scheuermann BW**, **Barstow TJ**. Effect of contraction frequency on leg blood flow during knee extension exercise in humans. *Journal of Applied Physiology* 91: 671–679, 2001. doi: 10.1152/jappl.2001.91.2.671.

102. **Green D**, **Cheetham C**, **Reed C**, **Dembo L**, **O'Driscoll G**. Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *Journal of Applied Physiology* 93: 361–368, 2002. doi: 10.1152/japplphysiol.00051.2002.

103. **Hunt JEA**, **Stodart C**, **Ferguson RA**. The influence of participant characteristics on the relationship between cuff pressure and level of blood flow restriction. *Eur J Appl Physiol* 116: 1421–1432, 2016. doi: 10.1007/s00421-016-3399-6.

104. **GAULT JH**, **ROSS J**, **MASON DT**. Patterns of Brachial Arterial Blood Flow in Conscious Human Subjects with and without Cardiac Dysfunction. *Circulation* 34: 833–848, 1966. doi: 10.1161/01.CIR.34.5.833.

105. **Trinity JD**, **Wray DW**, **Witman MAH**, **Layec G**, **Barrett-O'Keefe Z**, **Ives SJ**, **Conklin JD**, **Reese V**, **Richardson RS**. Contribution of nitric oxide to brachial artery vasodilation during progressive handgrip exercise in the elderly. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 305: R893–R899, 2013. doi: 10.1152/ajpregu.00311.2013.

106. **Calbet J a. L**, **González-Alonso J**, **Helge JW**, **Søndergaard H**, **Munch-Andersen T**, **Saltin B**, **Boushel R**. Central and peripheral hemodynamics in exercising humans: leg vs arm exercise. *Scandinavian Journal of Medicine & Science in Sports* 25: 144–157, 2015. doi: 10.1111/sms.12604.

107. **Hunter SK**, **Griffith EE**, **Schlachter KM**, **Kufahl TD**. Sex differences in time to task failure and blood flow for an intermittent isometric fatiguing contraction. *Muscle & Nerve* 39: 42–53, 2009. doi: 10.1002/mus.21203.

108. **Sheel AW**, **Derchak PA**, **Morgan BJ**, **Pegelow DF**, **Jacques AJ**, **Dempsey JA**. Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol* 537: 277–289, 2001. doi: 10.1111/j.1469-7793.2001.0277k.x.

109. **Otis AB**, **Fenn WO**, **Rahn H**. Mechanics of breathing in man. *J Appl Physiol* 2: 592–607, 1950. doi: 10.1152/jappl.1950.2.11.592.

110. **Otis AB**. The work of breathing. *Physiol Rev* 34: 449–458, 1954. doi: 10.1152/physrev.1954.34.3.449.

111. **Bird JD**, **Lance ML**, **Banser TRW**, **Thrall SF**, **Cotton PD**, **Lindner JR**, **Eves ND**, **Dominelli PB**, **Foster GE**. Quantifying Diaphragm Blood Flow With Contrast-Enhanced Ultrasound in Humans. *Chest* 166: 821–834, 2024. doi: 10.1016/j.chest.2024.04.026.

112. **Fogarty MJ**, **Sieck GC**. Evolution and Functional Differentiation of the Diaphragm Muscle of Mammals. *Compr Physiol* 9: 715–766, 2019. doi: 10.1002/cphy.c180012.

113. **Abel FL**, **Waldhausen JA**. Respiratory and cardiac effects on venous return. *American Heart Journal* 78: 266–275, 1969. doi: 10.1016/0002-8703(69)90019-2.

114. **Aaker A**, **Laughlin MH**. Diaphragm arterioles are less responsive to alpha1- adrenergic constriction than gastrocnemius arterioles. *J Appl Physiol* 92: 1808–1816, 2002. doi: 10.1152/japplphysiol.01152.2001.

115. **Tarasova OS**, **Kalenchuk VU**, **Borzykh AA**, **Andreev-Andrievskiĭ AA**, **Buravkov SV**, **Sharova AP**, **Vinogradova OL**. [Comparative analysis of vasomotor responses and sympathetic innervation of feed arteries from locomotor and respiratory muscles in a rat]. *Biofizika* 53: 1095–1101, 2008.

116. **Dominelli PB**, **Archiza B**, **Ramsook AH**, **Mitchell RA**, **Peters CM**, **Molgat-Seon Y**, **Henderson WR**, **Koehle MS**, **Boushel R**, **Sheel AW**. Effects of respiratory muscle work on respiratory and locomotor blood flow during exercise. *Exp Physiol* 102: 1535–1547, 2017. doi: 10.1113/EP086566.

117. **Dempsey JA**, **Sheel AW**, **St. Croix CM**, **Morgan BJ**. Respiratory influences on sympathetic vasomotor outflow in humans. *Respiratory Physiology & Neurobiology* 130: 3–20, 2002. doi: 10.1016/S0034-5687(01)00327-9.

118. **Dominelli PB**, **Katayama K**, **Vermeulen TD**, **Stuckless TJR**, **Brown CV**, **Foster GE**, **Sheel AW**. Work of breathing influences muscle sympathetic nerve activity during semi-recumbent cycle exercise. *Acta Physiol (Oxf)* 225: e13212, 2019. doi: 10.1111/apha.13212.

119. **Manohar M**. Blood flow to the respiratory and limb muscles and to abdominal organs during maximal exertion in ponies. *J Physiol* 377: 25–35, 1986. doi: 10.1113/jphysiol.1986.sp016174.

120. **Guenette JA**, **Henderson WR**, **Dominelli PB**, **Querido JS**, **Brasher PM**, **Griesdale DEG**, **Boushel R**, **Sheel AW**. Blood flow index using near-infrared spectroscopy and indocyanine green as a minimally invasive tool to assess respiratory muscle blood flow in humans. *Am J Physiol Regul Integr Comp Physiol* 300: R984-992, 2011. doi: 10.1152/ajpregu.00739.2010.

121. **Vogiatzis I**, **Louvaris Z**, **Wagner PD**. Respiratory and locomotor muscle blood flow during exercise in health and chronic obstructive pulmonary disease. *Experimental Physiology* 105: 1990–1996, 2020. doi: 10.1113/EP088104.

122. **Manohar M**. Costal vs. crural diaphragmatic blood flow during submaximal and near-maximal exercise in ponies. *J Appl Physiol (1985)* 65: 1514–1519, 1988. doi: 10.1152/jappl.1988.65.4.1514.

123. **Aaron EA**, **Seow KC**, **Johnson BD**, **Dempsey JA**. Oxygen cost of exercise hyperpnea: implications for performance. *J Appl Physiol (1985)* 72: 1818–1825, 1992. doi: 10.1152/jappl.1992.72.5.1818.

124. **Lewis T**, **Grant R**. Observations upon reactive hyperemia in man. *Heart* 12: 73–120, 1925.

125. **Joyner MJ**, **Dietz NM**, **Shepherd JT**. From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol (1985)* 91: 2431–2441, 2001. doi: 10.1152/jappl.2001.91.6.2431.

126. **Wahren J**, **Jorfeldt L**. Determination of leg blood flow during exercise in man: an indicator-dilution technique based on femoral venous dye infusion. *Clin Sci Mol Med* 45: 135–146, 1973. doi: 10.1042/cs0450135.

127. **Gliemann L**, **Mortensen SP**, **Hellsten Y**. Methods for the determination of skeletal muscle blood flow: development, strengths and limitations. *Eur J Appl Physiol* 118: 1081–1094, 2018. doi: 10.1007/s00421-018-3880-5.

128. **Ganz V**, **Hlavová A**, **Froněk A**, **Linhart J**, **Přerovský I**. Measurement of Blood Flow in the Femoral Artery in Man at Rest and during Exercise by Local Thermodilution. *Circulation* 30: 86–89, 1964. doi: 10.1161/01.CIR.30.1.86.

129. **Shoemaker JK**, **Pozeg ZI**, **Hughson RL**. Forearm blood flow by Doppler ultrasound during test and exercise: tests of day-to-day repeatability. *Med Sci Sports Exerc* 28: 1144–1149, 1996. doi: 10.1097/00005768-199609000-00010.

130. **Manohar M**. Diaphragmatic perfusion heterogeneity during exercise with inspiratory resistive breathing. *J Appl Physiol (1985)* 68: 2177–2181, 1990. doi: 10.1152/jappl.1990.68.5.2177.

131. **Manohar M**. Inspiratory and expiratory muscle perfusion in maximally exercised ponies. *J Appl Physiol (1985)* 68: 544–548, 1990. doi: 10.1152/jappl.1990.68.2.544.

132. **Robertson CH**, **Pagel MA**, **Johnson RL**. The distribution of blood flow, oxygen consumption, and work output among the respiratory muscles during unobstructed hyperventilation. *J Clin Invest* 59: 43–50, 1977. doi: 10.1172/JCI108620.

133. **Robertson CH**, **Eschenbacher WL**, **Johnson RL**. Respiratory muscle blood flow distribution during expiratory resistance. *J Clin Invest* 60: 473–480, 1977. doi: 10.1172/JCI108798.

134. **Rochester DF**. Measurement of diaphragmatic blood flow and oxygen consumption in the dog by the Kety-Schmidt technique. *J Clin Invest* 53: 1216–1225, 1974. doi: 10.1172/JCI107668.

135. **Rochester DF**, **Bettini G**. Diaphragmatic blood flow and energy expenditure in the dog. Effects of inspiratory airflow resistance and hypercapnia. *J Clin Invest* 57: 661–672, 1976.

136. **Rochester DF**, **Pradel-Guena M**. Measurement of diaphragmatic blood flow in dogs from xenon 133 clearance. *J Appl Physiol* 34: 68–74, 1973. doi: 10.1152/jappl.1973.34.1.68.

137. **Lance ML**, **Bruce CD**, **Coyle MM**, **Thrall SF**, **Bird JD**, **Witt PA**, **Dominelli PB**, **McNeil CJ**, **Foster GE**. The hyperaemic and metaboreflex response of the diaphragm during fatiguing diaphragmatic work in humans. *J Physiol* 603: 5805–5825, 2025. doi: 10.1113/JP288837.

138. **Aubier M**, **Murciano D**, **Menu Y**, **Boczkowski J**, **Mal H**, **Pariente R**. Dopamine effects on diaphragmatic strength during acute respiratory failure in chronic obstructive pulmonary disease. *Ann Intern Med* 110: 17–23, 1989. doi: 10.7326/0003-4819-110-1-17.

139. **de Bisschop C**, **Montaudon M**, **Glénet S**, **Guénard H**. Feasibility of intercostal blood flow measurement by echo-Doppler technique in healthy subjects. *Clinical Physiology and Functional Imaging* 37: 282–287, 2017. doi: 10.1111/cpf.12298.

140. **Louvaris Z**, **Rodrigues A**, **Dacha S**, **Gojevic T**, **Janssens W**, **Vogiatzis I**, **Gosselink R**, **Langer D**. High-intensity exercise impairs extradiaphragmatic respiratory muscle perfusion

in patients with COPD. *J Appl Physiol (1985)* 130: 325–341, 2021. doi: 10.1152/japplphysiol.00659.2020.

141. **Athanasopoulos D**, **Louvaris Z**, **Cherouveim E**, **Andrianopoulos V**, **Roussos C**, **Zakynthinos S**, **Vogiatzis I**. Expiratory muscle loading increases intercostal muscle blood flow during leg exercise in healthy humans. *J Appl Physiol (1985)* 109: 388–395, 2010. doi: 10.1152/japplphysiol.01290.2009.

142. **Vogiatzis I**, **Athanasopoulos D**, **Habazettl H**, **Kuebler WM**, **Wagner H**, **Roussos C**, **Wagner PD**, **Zakynthinos S**. Intercostal muscle blood flow limitation in athletes during maximal exercise. *J Physiol* 587: 3665–3677, 2009. doi: 10.1113/jphysiol.2009.171694.

143. **Molgat-Seon Y**, **Dominelli PB**, **Ramsook AH**, **Schaeffer MR**, **Romer LM**, **Road JD**, **Guenette JA**, **Sheel AW**. Effects of Age and Sex on Inspiratory Muscle Activation Patterns during Exercise. *Medicine & Science in Sports & Exercise* 50: 1882–1891, 2018. doi: 10.1249/MSS.0000000000001648.

144. **Aslan SC**, **McKay WB**, **Singh G**, **Ovechkin AV**. Respiratory muscle activation patterns during maximum airway pressure efforts are different in women and men. *Respir Physiol Neurobiol* 259: 143–148, 2019. doi: 10.1016/j.resp.2018.09.004.

145. **Shephard RJ**. Responses of the human spleen to exercise. *J Sports Sci* 34: 929–936, 2016. doi: 10.1080/02640414.2015.1078488.

146. **Fixler DE**, **Atkins JM**, **Mitchell JH**, **Horwitz LD**. Blood flow to respiratory, cardiac, and limb muscles in dogs during graded exercise. *Am J Physiol* 231: 1515–1519, 1976. doi: 10.1152/ajplegacy.1976.231.5.1515.

147. **Wei K**. Assessment of myocardial blood flow and volume using myocardial contrast echocardiography. *Echocardiography* 19: 409–416, 2002. doi: 10.1046/j.1540-8175.2002.00409.x.

148. **Wei K**, **Jayaweera AR**, **Firoozan S**, **Linka A**, **Skyba DM**, **Kaul S**. Quantification of Myocardial Blood Flow With Ultrasound-Induced Destruction of Microbubbles Administered as a Constant Venous Infusion. *Circulation* 97: 473–483, 1998. doi: 10.1161/01.CIR.97.5.473.

149. **Lindner JR**, **Firschke C**, **Wei K**, **Goodman NC**, **Skyba DM**, **Kaul S**. Myocardial perfusion characteristics and hemodynamic profile of MRX-115, a venous echocardiographic contrast agent, during acute myocardial infarction. *J Am Soc Echocardiogr* 11: 36–46, 1998. doi: 10.1016/s0894-7317(98)70118-x.

150. **Krix M**, **Plathow C**, **Essig M**, **Herfarth K**, **Debus J**, **Kauczor H-U**, **Delorme S**. Monitoring of liver metastases after stereotactic radiotherapy using low-MI contrast-enhanced ultrasound—initial results. *Eur Radiol* 15: 677–684, 2005. doi: 10.1007/s00330-004-2620-x.

151. **Krix M**, **Weber M-A**, **Kauczor H-U**, **Delorme S**, **Krakowski-Roosen H**. Changes in the micro-circulation of skeletal muscle due to varied isometric exercise assessed by contrast-enhanced ultrasound. *European Journal of Radiology* 76: 110–116, 2010. doi: 10.1016/j.ejrad.2009.05.007.

152. **Krix M**, **Krakowski-Roosen H**, **Kauczor H-U**, **Delorme S**, **Weber M-A**. Real-Time Contrast-Enhanced Ultrasound for the Assessment of Perfusion Dynamics in Skeletal Muscle. *Ultrasound in Medicine & Biology* 35: 1587–1595, 2009. doi: 10.1016/j.ultrasmedbio.2009.05.006.

153. **Davidson BP**, **Hodovan J**, **Belcik JT**, **Moccetti F**, **Xie A**, **Ammi AY**, **Lindner JR**. REST-STRESS LIMB PERFUSION IMAGING IN HUMANS WITH CONTRAST ULTRASOUND USING INTERMEDIATE-POWER IMAGING AND MICROBUBBLES RESISTANT TO INERTIAL CAVITATION. *J Am Soc Echocardiogr* 30: 503-510.e1, 2017. doi: 10.1016/j.echo.2016.12.011.

154. **Nguyen T**, **Davidson BP**. Contrast Enhanced Ultrasound Perfusion Imaging in Skeletal Muscle. *J Cardiovasc Imaging* 27: 163–177, 2019. doi: 10.4250/jcvi.2019.27.e31.

155. **Dawson D**, **Vincent MA**, **Barrett EJ**, **Kaul S**, **Clark A**, **Leong-Poi H**, **Lindner JR**. Vascular recruitment in skeletal muscle during exercise and hyperinsulinemia assessed by contrast ultrasound. *American Journal of Physiology-Endocrinology and Metabolism* 282: E714–E720, 2002. doi: 10.1152/ajpendo.00373.2001.

156. **Wilkinson IB**, **Webb DJ**. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol* 52: 631–646, 2001. doi: 10.1046/j.1365-2125.2001.01495.x.

157. **Molgat-Seon Y**, **Dominelli PB**, **Ramsook AH**, **Schaeffer MR**, **Molgat Sereacki S**, **Foster GE**, **Romer LM**, **Road JD**, **Guenette JA**, **Sheel AW**. The effects of age and sex on mechanical ventilatory constraint and dyspnea during exercise in healthy humans. *J Appl Physiol* 124: 1092–1106, 2018. doi: 10.1152/japplphysiol.00608.2017.

158. **Ripoll Sanz J**, **Guo W**, **Andersen K j.**, **Wiggins C c.**, **Baker S e.**, **Shepherd J-R a.**, **Carter R e.**, **Welch B t.**, **Joyner M j.**, **Dominelli P b.** Pediatric Sex-Differences in Large Conducting Airway Anatomy. In: *A67. STRUCTURE-FUNCTION RELATIONSHIPS*. American Thoracic Society, p. A2348–A2348.

159. **Nokoff NJ**, **Senefeld J**, **Krausz C**, **Hunter S**, **Joyner M**. Sex Differences in Athletic Performance: Perspectives on Transgender Athletes. *Exercise and Sport Sciences Reviews* 51: 85, 2023. doi: 10.1249/JES.0000000000000317.

160. **Murphy WG**. The sex difference in haemoglobin levels in adults - mechanisms, causes, and consequences. *Blood Rev* 28: 41–47, 2014. doi: 10.1016/j.blre.2013.12.003.

161. **Reed BG**, **Carr BR**. The Normal Menstrual Cycle and the Control of Ovulation [Online]. In: *Endotext*, edited by Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, de Herder WW, Dhatariya K, Dungan K, Hofland J, Kalra S, Kaltsas G, Kapoor

N, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, New M, Purnell J, Sahay R, Shah AS, Singer F, Sperling MA, Stratakis CA, Trence DL, Wilson DP. MDText.com, Inc. http://www.ncbi.nlm.nih.gov/books/NBK279054/ [18 Aug. 2023].

162. **dos Santos RL**, **da Silva FB**, **Ribeiro RF**, **Stefanon I**. Sex hormones in the cardiovascular system. *Horm Mol Biol Clin Investig* 18: 89–103, 2014. doi: 10.1515/hmbci-2013-0048.

163. **Selles J**, **Polini N**, **Alvarez C**, **Massheimer V**. Progesterone and 17 beta-estradiol acutely stimulate nitric oxide synthase activity in rat aorta and inhibit platelet aggregation. *Life Sci* 69: 815–827, 2001. doi: 10.1016/s0024-3205(01)01174-2.

164. **Wassmann K**, **Wassmann S**, **Nickenig G**. Progesterone antagonizes the vasoprotective effect of estrogen on antioxidant enzyme expression and function. *Circ Res* 97: 1046–1054, 2005. doi: 10.1161/01.RES.0000188212.57180.55.

165. **MacNutt MJ**, **De Souza MJ**, **Tomczak SE**, **Homer JL**, **Sheel AW**. Resting and exercise ventilatory chemosensitivity across the menstrual cycle. *Journal of Applied Physiology* 112: 737–747, 2012. doi: 10.1152/japplphysiol.00727.2011.

166. **Xue B**, **Singh M**, **Guo F**, **Hay M**, **Johnson AK**. Protective actions of estrogen on angiotensin II-induced hypertension: role of central nitric oxide. *Am J Physiol Heart Circ Physiol* 297: H1638–H1646, 2009. doi: 10.1152/ajpheart.00502.2009.

167. **Ciriello J**, **Roder S**. 17β-Estradiol alters the response of subfornical organ neurons that project to supraoptic nucleus to plasma angiotensin II and hypernatremia. *Brain Res* 1526: 54–64, 2013. doi: 10.1016/j.brainres.2013.06.038.

168. **Green DJ**, **Hopkins ND**, **Jones H**, **Thijssen DHJ**, **Eijsvogels TMH**, **Yeap BB**. Sex differences in vascular endothelial function and health in humans: impacts of exercise. *Exp Physiol* 101: 230–242, 2016. doi: 10.1113/EP085367.

169. **Ferrer M**, **Meyer M**, **Osol G**. Estrogen replacement increases beta-adrenoceptor-mediated relaxation of rat mesenteric arteries. *J Vasc Res* 33: 124–131, 1996. doi: 10.1159/000159140.

170. **Sudhir K**, **Jennings GL**, **Funder JW**, **Komesaroff PA**. Estrogen enhances basal nitric oxide release in the forearm vasculature in perimenopausal women. *Hypertension* 28: 330–334, 1996. doi: 10.1161/01.hyp.28.3.330.

171. **Olivetti G**, **Giordano G**, **Corradi D**, **Melissari M**, **Lagrasta C**, **Gambert SR**, **Anversa P**. Gender differences and aging: Effects on the human heart. *Journal of the American College of Cardiology* 26: 1068–1079, 1995. doi: 10.1016/0735-1097(95)00282-8.

172. **Bredella MA**. Sex Differences in Body Composition. In: *Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity*, edited by Mauvais-Jarvis F. Springer International Publishing, p. 9–27.

173. **Cruickshank JM**. Some Variations in the Normal Haemoglobin Concentration. *British Journal of Haematology* 18: 523–530, 1970. doi: 10.1111/j.1365-2141.1970.tb00773.x.

174. **Skattebo Ø**, **Martin-Rincon M**, **Rud B**, **Nielsen J**, **Hegg LHN**, **Kleive AV**, **Ørtenblad N**, **Sandbakk Ø**, **Boushel R**, **Holmberg H-C**, **Calbet JAL**, **Hallén J**. Determinants of maximal oxygen uptake in highly trained females and males: a mechanistic study of sex differences using advanced invasive methods. .

175. **DuPont JJ**, **Kenney RM**, **Patel AR**, **Jaffe IZ**. Sex differences in mechanisms of arterial stiffness. *Br J Pharmacol* 176: 4208–4225, 2019. doi: 10.1111/bph.14624.

176. **Rylski B**, **Desjardins B**, **Moser W**, **Bavaria JE**, **Milewski RK**. Gender-related changes in aortic geometry throughout life†. *European Journal of Cardio-Thoracic Surgery* 45: 805–811, 2014. doi: 10.1093/ejcts/ezt597.

177. **Sandgren T**, **Sonesson B**, **Ahlgren R**, **Länne T**. The diameter of the common femoral artery in healthy human: influence of sex, age, and body size. *J Vasc Surg* 29: 503–510, 1999. doi: 10.1016/s0741-5214(99)70279-x.

178. **Hopkins ND**, **Dengel DR**, **Stratton G**, **Kelly AS**, **Steinberger J**, **Zavala H**, **Marlatt K**, **Perry D**, **Naylor LH**, **Green DJ**. Age and sex relationship with flow-mediated dilation in healthy children and adolescents. *J Appl Physiol (1985)* 119: 926–933, 2015. doi: 10.1152/japplphysiol.01113.2014.

179. **Tremblay JC**, **Stimpson TV**, **Pyke KE**. Evidence of sex differences in the acute impact of oscillatory shear stress on endothelial function. *J Appl Physiol (1985)* 126: 314–321, 2019. doi: 10.1152/japplphysiol.00729.2018.

180. **Charkoudian N**, **Joyner M**, **Johnson C**, **Eisenach J**, **Dietz N**, **Wallin B**. Balance between cardiac output and sympathetic nerve activity in resting humans: role in arterial pressure regulation. *J Physiol* 568: 315–321, 2005. doi: 10.1113/jphysiol.2005.090076.

181. **Hart EC**, **Charkoudian N**, **Wallin BG**, **Curry TB**, **Eisenach JH**, **Joyner MJ**. Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertens* 53: 571–576, 2009. doi: 10.1161/HYPERTENSIONAHA.108.126391.

182. **Riedel K**, **Deussen AJ**, **Tolkmitt J**, **Weber S**, **Schlinkert P**, **Zatschler B**, **Friebel C**, **Müller B**, **El-Armouche A**, **Morawietz H**, **Matschke K**, **Kopaliani I**. Estrogen determines sex differences in adrenergic vessel tone by regulation of endothelial β-adrenoceptor expression. *American Journal of Physiology-Heart and Circulatory Physiology* 317: H243–H254, 2019. doi: 10.1152/ajpheart.00456.2018.

183. **Saito Y**, **Iemitsu M**, **Otsuki T**, **Maeda S**, **Ajisaka R**. Gender differences in brachial blood flow during fatiguing intermittent handgrip. *Med Sci Sports Exerc* 40: 684–690, 2008. doi: 10.1249/MSS.0b013e3181614327.

184. **Thompson BC**, **Fadia T**, **Pincivero DM**, **Scheuermann BW**. Forearm blood flow responses to fatiguing isometric contractions in women and men. *Am J Physiol Heart Circ Physiol* 293: H805-812, 2007. doi: 10.1152/ajpheart.01136.2006.

185. **Kellawan JM**, **Johansson RE**, **Harrell JW**, **Sebranek JJ**, **Walker BJ**, **Eldridge MW**, **Schrage WG**. Exercise vasodilation is greater in women: contributions of nitric oxide synthase and cyclooxygenase. *Eur J Appl Physiol* 115: 1735–1746, 2015. doi: 10.1007/s00421-015-3160-6.

186. **Hill EC**, **Housh TJ**, **Keller JL**, **Smith CM**, **Schmidt RJ**, **Johnson GO**. Sex differences for fatigue-induced changes in muscle blood flow, but not eccentric peak torque or neuromuscular responses. *J Musculoskelet Neuronal Interact* 18: 427–437, 2018.

187. **Fulco CS**, **Lewis SF**, **Frykman PN**, **Boushel R**, **Smith S**, **Harman EA**, **Cymerman A**, **Pandolf KB**. Muscle fatigue and exhaustion during dynamic leg exercise in normoxia and hypobaric hypoxia. *Journal of Applied Physiology* 81: 1891–1900, 1996. doi: 10.1152/jappl.1996.81.5.1891.

188. **Labarbera KE**, **Murphy BG**, **Laroche DP**, **Cook SB**. Sex differences in blood flow restricted isotonic knee extensions to fatigue. *J Sports Med Phys Fitness* 53: 444–452, 2013.

189. **Hunter SK**. Sex Differences and Mechanisms of Task-Specific Muscle Fatigue. *Exerc Sport Sci Rev* 37: 113–122, 2009. doi: 10.1097/JES.0b013e3181aa63e2.

190. **Hunter SK**, **Critchlow A**, **Shin I-S**, **Enoka RM**. Men are more fatigable than strength-matched women when performing intermittent submaximal contractions. *J Appl Physiol (1985)* 96: 2125–2132, 2004. doi: 10.1152/japplphysiol.01342.2003.

191. **Mitchell RA**, **Schaeffer MR**, **Ramsook AH**, **Wilkie SS**, **Guenette JA**. Sex differences in respiratory muscle activation patterns during high-intensity exercise in healthy humans. *Respir Physiol Neurobiol* 247: 57–60, 2018. doi: 10.1016/j.resp.2017.09.002.

192. **Guenette JA**, **Romer LM**, **Querido JS**, **Chua R**, **Eves ND**, **Road JD**, **McKenzie DC**, **Sheel AW**. Sex differences in exercise-induced diaphragmatic fatigue in endurance-trained athletes. *J Appl Physiol (1985)* 109: 35–46, 2010. doi: 10.1152/japplphysiol.01341.2009.

193. **Bellemare F**, **Jeanneret A**, **Couture J**. Sex differences in thoracic dimensions and configuration. *Am J Respir Crit Care Med* 168: 305–312, 2003. doi: 10.1164/rccm.200208-876OC.

194. **Schwartz J**, **Katz SA**, **Fegley RW**, **Tockman MS**. Sex and race differences in the development of lung function. *Am Rev Respir Dis* 138: 1415–1421, 1988. doi: 10.1164/ajrccm/138.6.1415.

195. **Crapo RO**, **Morris AH**, **Clayton PD**, **Nixon CR**. Lung volumes in healthy nonsmoking adults. *Bull Eur Physiopathol Respir* 18: 419–425, 1982.

196. **García-Martínez D**, **Torres-Tamayo N**, **Torres-Sanchez I**, **García-Río F**, **Bastir M**. Morphological and functional implications of sexual dimorphism in the human skeletal thorax. *Am J Phys Anthropol* 161: 467–477, 2016. doi: 10.1002/ajpa.23051.

197. **Torres-Tamayo N**, **García-Martínez D**, **Lois Zlolniski S**, **Torres-Sánchez I**, **García-Río F**, **Bastir M**. 3D analysis of sexual dimorphism in size, shape and breathing kinematics of human lungs. *J Anat* 232: 227–237, 2018. doi: 10.1111/joa.12743.

198. **Mead J**. Dysanapsis in normal lungs assessed by the relationship between maximal flow, static recoil, and vital capacity. *Am Rev Respir Dis* 121: 339–342, 1980. doi: 10.1164/arrd.1980.121.2.339.

199. **Dominelli PB**, **Molgat-Seon Y**, **Bingham D**, **Swartz PM**, **Road JD**, **Foster GE**, **Sheel AW**. Dysanapsis and the resistive work of breathing during exercise in healthy men and women. *J Appl Physiol* 119: 1105–1113, 2015. doi: 10.1152/japplphysiol.00409.2015.

200. **Breatnach E**, **Abbott GC**, **Fraser RG**. Dimensions of the normal human trachea. *AJR Am J Roentgenol* 142: 903–906, 1984. doi: 10.2214/ajr.142.5.903.

201. **Martin TR**, **Castile RG**, **Fredberg JJ**, **Wohl ME**, **Mead J**. Airway size is related to sex but not lung size in normal adults. *J Appl Physiol (1985)* 63: 2042–2047, 1987. doi: 10.1152/jappl.1987.63.5.2042.

202. **Peters CM**, **Leahy MG**, **Hohert G**, **Lane P**, **Lam S**, **Sin DD**, **McKenzie DC**, **Sheel AW**. Airway luminal area and the resistive work of breathing during exercise in healthy young females and males. *Journal of Applied Physiology* 131: 1750–1761, 2021. doi: 10.1152/japplphysiol.00418.2021.

203. **Dominelli PB**, **Ripoll JG**, **Cross TJ**, **Baker SE**, **Wiggins CC**, **Welch BT**, **Joyner MJ**. Sex differences in large conducting airway anatomy. *J Appl Physiol* 125: 960–965, 2018. doi: 10.1152/japplphysiol.00440.2018.

204. **Christou S**, **Chatziathanasiou T**, **Angeli S**, **Koullapis P**, **Stylianou F**, **Sznitman J**, **Guo HH**, **Kassinos SC**. Anatomical variability in the upper tracheobronchial tree: sex-based differences and implications for personalized inhalation therapies. *J Appl Physiol (1985)* 130: 678–707, 2021. doi: 10.1152/japplphysiol.00144.2020.

205. **Hackx M**, **Francotte D**, **Garcia TS**, **Van Muylem A**, **Walsdorff M**, **Gevenois PA**. Effect of total lung capacity, gender and height on CT airway measurements. *Br J Radiol* 90: 20160898, 2017. doi: 10.1259/bjr.20160898.

206. **Sheel AW**, **Guenette JA**, **Yuan R**, **Holy L**, **Mayo JR**, **McWilliams AM**, **Lam S**, **Coxson HO**. Evidence for dysanapsis using computed tomographic imaging of the airways in older ex-smokers. *J Appl Physiol* 107: 1622–1628, 2009. doi: 10.1152/japplphysiol.00562.2009.

207. **Knudson RJ**, **Lebowitz MD**, **Holberg CJ**, **Burrows B**. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis* 127: 725–734, 1983. doi: 10.1164/arrd.1983.127.6.725.

208. **Black LF**, **Hyatt RE**. Maximal respiratory pressures: normal values and relationship to age and sex. *Am Rev Respir Dis* 99: 696–702, 1969. doi: 10.1164/arrd.1969.99.5.696.

209. **Olfert IM**, **Balouch J**, **Kleinsasser A**, **Knapp A**, **Wagner H**, **Wagner PD**, **Hopkins SR**. Does gender affect human pulmonary gas exchange during exercise? *J Physiol* 557: 529–541, 2004. doi: 10.1113/jphysiol.2003.056887.

210. **Bouwsema MM**, **Tedjasaputra V**, **Stickland MK**. Are there sex differences in the capillary blood volume and diffusing capacity response to exercise? *J Appl Physiol (1985)* 122: 460–469, 2017. doi: 10.1152/japplphysiol.00389.2016.

211. **Dominelli PB**, **Render JN**, **Molgat-Seon Y**, **Foster GE**, **Romer LM**, **Sheel AW**. Oxygen cost of exercise hyperpnoea is greater in women compared with men. *J Physiol* 593: 1965–1979, 2015. doi: 10.1113/jphysiol.2014.285965.

212. **Mann LM**, **Granger EA**, **Chan JS**, **Yu A**, **Molgat-Seon Y**, **Dominelli PB**. Minimizing airflow turbulence in women lowers the work of breathing to levels similar to men. *J Appl Physiol (1985)* 129: 410–418, 2020. doi: 10.1152/japplphysiol.00347.2020.

213. **Eckermann P**, **Millahn HP**. [Oxygen consumption of the respiratory musculature in women. With an observation on the efficiency of the respiratory musculature]. *Int Z Angew Physiol* 19: 168–172, 1962.

214. **Ogrodowczyk M**, **Dettlaff K**, **Jelinska A**. Beta-Blockers: Current State of Knowledge and Perspectives. *Mini Rev Med Chem* 16: 40–54, 2016. doi: 10.2174/1389557515666151016125948.

215. **Tattersfield AE**, **Leaver DG**, **Pride NB**. Effects of beta-adrenergic blockade and stimulation on normal human airways. *J Appl Physiol* 35: 613–619, 1973. doi: 10.1152/jappl.1973.35.5.613.

216. **Bell C**, **Seals DR**, **Monroe MB**, **Day DS**, **Shapiro LF**, **Johnson DG**, **Jones PP**. Tonic sympathetic support of metabolic rate is attenuated with age, sedentary lifestyle, and female sex in healthy adults. *J Clin Endocrinol Metab* 86: 4440–4444, 2001. doi: 10.1210/jcem.86.9.7855.

217. **Hughson RL**, **MacFarlane BJ**. Effect of oral propranolol on the anerobic threshold and maximum exercise performance in normal man. *Can J Physiol Pharmacol* 59: 567–573, 1981. doi: 10.1139/y81-085.

218. **Ekblom B**, **Goldbarg AN**, **Kilbom A**, **Astrand PO**. Effects of atropine and propranolol on the oxygen transport system during exercise in man. *Scand J Clin Lab Invest* 30: 35–42, 1972. doi: 10.3109/00365517209081087.

219. **Nies AS**, **Shand DG**. Clinical pharmacology of propranolol. *Circulation* 52: 6–15, 1975. doi: 10.1161/01.cir.52.1.6.

220. **Shand DG**, **Nuckolls EM**, **Oates JA**. Plasma propranolol levels in adults With observations in four children. *Clinical Pharmacology & Therapeutics* 11: 112–120, 1970. doi: 10.1002/cpt1970111112.

221. **Bodem G**, **Brammell HL**, **Weil JV**, **Chidsey CA**. Pharmacodynamic Studies of Beta Adrenergic Antagonism Induced in Man by Propranolol and Practolol. *J Clin Invest* 52: 747–754, 1973. doi: 10.1172/JCI107237.

222. Product monograph intravenous propranolol hydrochloride [Online]. Sandoz Canada Inc.: 2013. https://www.sandoz.ca/sites/www.sandoz.ca/files/Propranolol%20HCl%20PMe%20%2020130619.pdf.

223. **Paterson JW**, **Conolly ME**, **Dollery CT**, **Hayes A**, **Cooper RG**. The pharmacodynamics and metabolism of propranolol in man. *Pharmacol Clin* 2: 127–133, 1970. doi: 10.1007/BF00420338.

224. **Evans GH**, **Shand DG**. Disposition of propranolol V. Drug accumulation and steady-state concentrations during chronic oral administration in man. *Clinical Pharmacology & Therapeutics* 14: 487–493, 1973. doi: 10.1002/cpt1973144part1487.

225. **Vervloet E**, **Pluym BFM**, **Cilissen J**, **Kahlen K**, **Merkus FWHM**. Propranolol serum levels during twenty-four hours. *Clinical Pharmacology & Therapeutics* 22: 853–857, 1977. doi: 10.1002/cpt1977226853.

226. **Jefferson D**, **Jenner P**, **Marsden CD**. Relationship between plasma propranolol concentration and relief of essential tremor. *J Neurol Neurosurg Psychiatry* 42: 831–837, 1979. doi: 10.1136/jnnp.42.9.831.

227. **Monroe MB**, **Seals DR**, **Shapiro LF**, **Bell C**, **Johnson D**, **Jones PP**. Direct evidence for tonic sympathetic support of resting metabolic rate in healthy adult humans. *American Journal of Physiology-Endocrinology and Metabolism* 280: E740–E744, 2001. doi: 10.1152/ajpendo.2001.280.5.E740.

228. **Casey DP**, **Curry TB**, **Joyner MJ**, **Charkoudian N**, **Hart EC**. Acute β-adrenergic blockade increases aortic wave reflection in young men and women: differing mechanisms between sexes. *Hypertension* 59: 145–150, 2012. doi: 10.1161/HYPERTENSIONAHA.111.182337.

229. **Crawford MH**, **Lindenfeld J**, **O'Rourke RA**. Effects of oral propranolol on left ventricular size and performance during exercise and acute pressure loading. *Circulation* 61: 549–554, 1980. doi: 10.1161/01.cir.61.3.549.

230. **Bruce RA**, **Hossack HF**, **Kusumi F**, **Clarke LJ**. Acute effects of oral propranolol on hemodynamic responses to upright exercise. *Am J Cardiol* 44: 132–140, 1979. doi: 10.1016/0002-9149(79)90262-5.

231. **Maman SR**, **Vargas AF**, **Ahmad TA**, **Miller AJ**, **Gao Z**, **Leuenberger UA**, **Proctor DN**, **Muller MD**. Beta-1 vs. beta-2 adrenergic control of coronary blood flow during isometric handgrip exercise in humans. *J Appl Physiol (1985)* 123: 337–343, 2017. doi: 10.1152/japplphysiol.00106.2017.

232. **Ishii K**, **Matsukawa K**, **Liang N**, **Endo K**, **Idesako M**, **Hamada H**, **Kataoka T**, **Ueno K**, **Watanabe T**, **Takahashi M**. Differential contribution of ACh-muscarinic and β-adrenergic receptors to vasodilatation in noncontracting muscle during voluntary one-legged exercise. *Physiol Rep* 2: e12202, 2014. doi: 10.14814/phy2.12202.

233. **Casey DP**, **Curry TB**, **Wilkins BW**, **Joyner MJ**. Nitric oxide-mediated vasodilation becomes independent of beta-adrenergic receptor activation with increased intensity of hypoxic exercise. *J Appl Physiol (1985)* 110: 687–694, 2011. doi: 10.1152/japplphysiol.00787.2010.

234. **Ross AJ**, **Gao Z**, **Pollock JP**, **Leuenberger UA**, **Sinoway LI**, **Muller MD**. β-Adrenergic receptor blockade impairs coronary exercise hyperemia in young men but not older men. *Am J Physiol Heart Circ Physiol* 307: H1497-1503, 2014. doi: 10.1152/ajpheart.00584.2014.

235. **Pellinger TK**, **Halliwill JR**. Effect of propranolol on sympathetically mediated leg vasoconstriction in humans. *J Physiol* 583: 797–809, 2007. doi: 10.1113/jphysiol.2007.137422.

236. **Epstein S**, **Robinson BF**, **Kahler RL**, **Braunwald E**. Effects of beta-adrenergic blockade on the cardiac response to maximal and submaximal exercise in man. *J Clin Invest* 44: 1745–1753, 1965.

237. **Seifert T**, **Rasmussen P**, **Secher NH**, **Nielsen HB**. Cerebral oxygenation decreases during exercise in humans with beta-adrenergic blockade. *Acta Physiol (Oxf)* 196: 295–302, 2009. doi: 10.1111/j.1748-1716.2008.01946.x.

238. **MacFarlane BJ**, **Hughson RL**, **Green HJ**, **Walters DJ**, **Ranney DA**. Effects of oral propranolol and exercise protocol on indices of aerobic function in normal man. *Can J Physiol Pharmacol* 61: 1010–1016, 1983. doi: 10.1139/y83-151.

239. **Marshall RC**, **Wisenberg G**, **Schelbert HR**, **Henze E**. Effect of oral propranolol on rest, exercise and postexercise left ventricular performance in normal subjects and patients with coronary artery disease. *Circulation* 63: 572–583, 1981. doi: 10.1161/01.cir.63.3.572.

240. **Canning BJ**. Reflex regulation of airway smooth muscle tone. *Journal of Applied Physiology* 101: 971–985, 2006. doi: 10.1152/japplphysiol.00313.2006.

241. **Berkin KE**, **Walker G**, **Thomson NC**. Evidence for a dual effect by beta-adrenoceptor antagonists on post-exercise airway calibre. *European Respiratory Journal* 1: 227–231, 1988. doi: 10.1183/09031936.93.01030227.

242. **Richardson PS**, **Sterling GM**. Effects of β-Adrenergic Receptor Blockade on Airway Conductance and Lung Volume in Normal and Asthmatic Subjects. *Br Med J* 3: 143–145, 1969.

243. **Macdonald AG**, **Ingram CG**, **McNeill RS**. The effect of propranolol on airway resistance. *Br J Anaesth* 39: 919–926, 1967. doi: 10.1093/bja/39.12.919.

244. **Berkin KE**, **Inglis GC**, **Ball SG**, **Thomson NC**. Airway responses to low concentrations of adrenaline and noradrenaline in normal subjects. *Q J Exp Physiol* 70: 203–209, 1985. doi: 10.1113/expphysiol.1985.sp002903.

245. **Antonelli A**, **Torchio R**, **Bertolaccini L**, **Terzi A**, **Rolfo F**, **Agostoni P**, **Gulotta C**, **Brusasco V**, **Pellegrino R**. Contribution of β-adrenergic receptors to exercise-induced bronchodilatation in healthy humans. *Respiratory Physiology & Neurobiology* 184: 55–59, 2012. doi: 10.1016/j.resp.2012.07.007.

246. **Coltart DJ**, **Shand DG**. Plasma Propranolol Levels in the Quantitative Assessment of β-adrenergic Blockade in Man. *Br Med J* 3: 731–734, 1970.

247. **Hillig T**, **Krustrup P**, **Fleming I**, **Osada T**, **Saltin B**, **Hellsten Y**. Cytochrome P450 2C9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. *J Physiol* 546: 307–314, 2003. doi: 10.1113/jphysiol.2002.030833.

248. **Lamb IR**, **Novielli NM**, **Murrant CL**. Capillary response to skeletal muscle contraction: evidence that redundancy between vasodilators is physiologically relevant during active hyperaemia. *J Physiol* 596: 1357–1372, 2018. doi: 10.1113/JP275467.

249. **Saltin B**. Exercise hyperaemia: magnitude and aspects on regulation in humans. *J Physiol* 583: 819–823, 2007. doi: 10.1113/jphysiol.2007.136309.

250. **Burt VL**, **Whelton P**, **Roccella EJ**, **Brown C**, **Cutler JA**, **Higgins M**, **Horan MJ**, **Labarthe D**. Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988-1991. *Hypertension* 25: 305–313, 1995. doi: 10.1161/01.hyp.25.3.305.

251. **Wiinberg N**, **Høegholm A**, **Christensen HR**, **Bang LE**, **Mikkelsen KL**, **Nielsen PE**, **Svendsen TL**, **Kampmann JP**, **Madsen NH**, **Bentzon MW**. 24-h ambulatory blood pressure in 352 normal Danish subjects, related to age and gender. *Am J Hypertens* 8: 978–986, 1995. doi: 10.1016/0895-7061(95)00216-2.

252. **Limberg JK**, **Eldridge MW**, **Proctor LT**, **Sebranek JJ**, **Schrage WG**. α-Adrenergic control of blood flow during exercise: effect of sex and menstrual phase. *Journal of Applied Physiology* 109: 1360–1368, 2010. doi: 10.1152/japplphysiol.00518.2010.

253. **Joyner MJ**, **Wallin BG**, **Charkoudian N**. Sex differences and blood pressure regulation in humans. *Experimental Physiology* 101: 349–355, 2016. doi: 10.1113/EP085146.

254. **Wilkins BW**, **Pike TL**, **Martin EA**, **Curry TB**, **Ceridon ML**, **Joyner MJ**. Exercise intensity-dependent contribution of β-adrenergic receptor-mediated vasodilatation in hypoxic humans. *The Journal of Physiology* 586: 1195–1205, 2008. doi: 10.1113/jphysiol.2007.144113.

255. **Nakatani M**, **Takai Y**, **Akagi R**, **Wakahara T**, **Sugisaki N**, **Ohta M**, **Kawakami Y**, **Fukunaga T**, **Kanehisa H**. Validity of muscle thickness-based prediction equation for quadriceps femoris volume in middle-aged and older men and women. *Eur J Appl Physiol* 116: 2125–2133, 2016. doi: 10.1007/s00421-016-3464-1.

256. **Thompson BP**, **Doherty CJ**, **Mann LM**, **Chang J-C**, **Angus SA**, **Foster GE**, **Au JS**, **Dominelli PB**. Supramaximal Testing to Confirm the Achievement of V̇O2max in Acute Hypoxia. *Medicine & Science in Sports & Exercise* 56: 673, 2024. doi: 10.1249/MSS.0000000000003339.

257. **Koo TK**, **Li MY**. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med* 15: 155–163, 2016. doi: 10.1016/j.jcm.2016.02.012.

258. **Wong Y-M**, **Ng GYF**. Surface electrode placement affects the EMG recordings of the quadriceps muscles. *Physical Therapy in Sport* 7: 122–127, 2006. doi: 10.1016/j.ptsp.2006.03.006.

259. **Sung JH**, **Baek S-H**, **Park J-W**, **Rho JH**, **Kim B-J**. Surface Electromyography-Driven Parameters for Representing Muscle Mass and Strength. *Sensors* 23: 5490, 2023. doi: 10.3390/s23125490.

260. **Gonzales JU**, **Thompson BC**, **Thistlethwaite JR**, **Harper AJ**, **Scheuermann BW**. Forearm blood flow follows work rate during submaximal dynamic forearm exercise independent of sex. *Journal of Applied Physiology* 103: 1950–1957, 2007. doi: 10.1152/japplphysiol.00452.2007.

261. **Koskolou MD**, **Calbet JA**, **Radegran G**, **Roach RC**. Hypoxia and the cardiovascular response to dynamic knee-extensor exercise. *American Journal of Physiology-Heart and Circulatory Physiology* 272: H2655–H2663, 1997. doi: 10.1152/ajpheart.1997.272.6.H2655.

262. **Roach RC**, **Koskolou MD**, **Calbet JAL**, **Saltin B**. Arterial O2 content and tension in regulation of cardiac output and leg blood flow during exercise in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 276: H438–H445, 1999. doi: 10.1152/ajpheart.1999.276.2.H438.

263. **Lutjemeier BJ**, **Miura A**, **Scheuermann BW**, **Koga S**, **Townsend DK**, **Barstow TJ**. Muscle contraction-blood flow interactions during upright knee extension exercise in humans. *J Appl Physiol (1985)* 98: 1575–1583, 2005. doi: 10.1152/japplphysiol.00219.2004.

264. **Huang A**, **Sun D**, **Koller A**, **Kaley G**. Gender difference in flow-induced dilation and regulation of shear stress: role of estrogen and nitric oxide. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 275: R1571–R1577, 1998. doi: 10.1152/ajpregu.1998.275.5.R1571.

265. **Joyner MJ**, **Barnes JN**, **Hart EC**, **Wallin BG**, **Charkoudian N**. Neural Control of the Circulation: How Sex and Age Differences Interact in Humans. *Compr Physiol* 5: 193–215, 2015. doi: 10.1002/cphy.c140005.

266. **Dawes M**, **Chowienczyk PJ**, **Ritter JM**. Effects of Inhibition of the l-Arginine/Nitric Oxide Pathway on Vasodilation Caused by β-Adrenergic Agonists in Human Forearm. *Circulation* 95: 2293–2297, 1997. doi: 10.1161/01.CIR.95.9.2293.

267. **Martin EA**, **Nicholson WT**, **Curry TB**, **Eisenach JH**, **Charkoudian N**, **Joyner MJ**. Adenosine transporter antagonism in humans augments vasodilator responsiveness to adenosine, but not exercise, in both adenosine responders and non-responders. *J Physiol* 579: 237–245, 2007. doi: 10.1113/jphysiol.2006.123000.

268. **Limberg JK**, **Johansson RE**, **Peltonen GL**, **Harrell JW**, **Kellawan JM**, **Eldridge MW**, **Sebranek JJ**, **Schrage WG**. β-Adrenergic-mediated vasodilation in young men and women: cyclooxygenase restrains nitric oxide synthase. *American Journal of Physiology - Heart and Circulatory Physiology* 310: H756, 2016. doi: 10.1152/ajpheart.00886.2015.

269. **Smith JR**, **Koepp KE**, **Berg JD**, **Akinsanya JG**, **Olson TP**. Influence of Sex, Menstrual Cycle, and Menopause Status on the Exercise Pressor Reflex. *Med Sci Sports Exerc* 51: 874–881, 2019. doi: 10.1249/MSS.0000000000001877.

270. **Andersen P**, **Adams RP**, **Sjøgaard G**, **Thorboe A**, **Saltin B**. Dynamic knee extension as model for study of isolated exercising muscle in humans. *J Appl Physiol (1985)* 59: 1647–1653, 1985. doi: 10.1152/jappl.1985.59.5.1647.

271. **Doherty CJ**, **Thompson BP**, **Rynne PJ**, **Chang J-C**, **Pulford-Thorpe AE**, **Angus SA**, **Fleming ECM**, **Gartner SL**, **Au JS**, **Foster GE**, **Dominelli PB**. The role of β-adrenergic receptors in mediating exercise hyperemia during incremental single-leg knee extension in young males and females. *Journal of Applied Physiology* 138: 1664–1679, 2025. doi: 10.1152/japplphysiol.00896.2024.

272. **Casey DP**, **Shepherd JRA**, **Joyner MJ**. Sex and vasodilator responses to hypoxia at rest and during exercise. *J Appl Physiol (1985)* 116: 927–936, 2014. doi: 10.1152/japplphysiol.00409.2013.

273. **Maier LE**, **Vanden Berg ER**, **Simpson L**, **Ewalts M**, **Foster K**, **Baylis J**, **Gasho C**, **Macleod D**, **van Diepen S**, **Anholm J**, **Lawley J**, **Ainslie PN**, **Gibbons TD**, **Stembridge M**, **Moore J**, **Steinback CD**. The effect of sex on the isolated and combined α- and β-adrenergic control of blood flow during handgrip in adults at high altitude: An exploratory study. *Physiol Rep* 14: e70754, 2026. doi: 10.14814/phy2.70754.

274. **Wargin WA**, **Sawchuk RJ**, **McBride JW**, **McCoy HG**, **Rylander ML**. Variable first-pass elimination of propranolol following single and multiple oral doses in hypertensive patients. *European Journal of Drug Metabolism and Pharmacokinetics* 7: 183–189, 1982. doi: 10.1007/BF03189564.

275. **Coltart DJ**, **Shand DG**. Plasma Propranolol Levels in the Quantitative Assessment of β-adrenergic Blockade in Man. *Br Med J* 3: 731–734, 1970. doi: 10.1136/bmj.3.5725.731.

276. **Vandongen R**, **Davidson L**, **Beilin LJ**, **Barden AE**. Effect of beta-adrenergic receptor blockade with propranolol on the response of plasma catecholamines and renin activity to upright tilting in normal subjects. *Br J Clin Pharmacol* 12: 369–374, 1981. doi: 10.1111/j.1365-2125.1981.tb01228.x.

277. **Coolbaugh CL**, **Bush EC**, **Caskey CF**, **Damon BM**, **Towse TF**. FloWave.US: validated, open-source, and flexible software for ultrasound blood flow analysis. *Journal of Applied Physiology* 121: 849–857, 2016. doi: 10.1152/japplphysiol.00819.2015.

278. **Koch DW**, **Newcomer SC**, **Proctor DN**. Blood Flow to Exercising Limbs Varies With Age, Gender, and Training Status. *Can J Appl Physiol* 30: 554–575, 2005. doi: 10.1139/h05-141.

279. **Perez J**, **Liu R**, **Dalve A**, **Nyarko R**, **Joergensen E**, **Dombroski L**, **Fatahimeiabadi Z**, **Kaur J**. Sex differences in forearm blood flow and vascular conductance responses to rhythmic handgrip exercise in young, healthy Hispanic/Latino adults. *Physiology* 39: 1926, 2024. doi: 10.1152/physiol.2024.39.S1.1926.

280. **Teixeira AL**, **Garland M**, **Lee JB**, **Nardone M**, **Millar PJ**. Assessing functional sympatholysis during rhythmic handgrip exercise using Doppler ultrasound and near-infrared spectroscopy: sex differences and test-retest reliability. *Am J Physiol Regul Integr Comp Physiol* 323: R810–R821, 2022. doi: 10.1152/ajpregu.00123.2022.

281. **Seals DR**, **Johnson DG**, **Fregosi RF**. Hypoxia potentiates exercise-induced sympathetic neural activation in humans. *Journal of Applied Physiology* 71: 1032–1040, 1991. doi: 10.1152/jappl.1991.71.3.1032.

282. **Hellsten Y**, **Nyberg M**, **Jensen LG**, **Mortensen SP**. Vasodilator interactions in skeletal muscle blood flow regulation. *The Journal of Physiology* 590: 6297–6305, 2012. doi: 10.1113/jphysiol.2012.240762.

283. **Sheel AW**, **Boushel R**, **Dempsey JA**. Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. *J Appl Physiol (1985)* 125: 820–831, 2018. doi: 10.1152/japplphysiol.00189.2018.

284. **Wanke T**, **Formanek D**, **Schenz G**, **Popp W**, **Gatol H**, **Zwick H**. Mechanical load on the ventilatory muscles during an incremental cycle ergometer test. *European Respiratory Journal* 4: 385–392, 1991.

285. **Chan JS**, **Mann LM**, **Doherty CJ**, **Angus SA**, **Thompson BP**, **Devries MC**, **Hughson RL**, **Dominelli PB**. The effect of inspiratory muscle training and detraining on the respiratory metaboreflex. *Experimental Physiology* 108: 636–649, 2023. doi: 10.1113/EP090779.

286. **Bowerman C**, **Bhakta NR**, **Brazzale D**, **Cooper BR**, **Cooper J**, **Gochicoa-Rangel L**, **Haynes J**, **Kaminsky DA**, **Lan LTT**, **Masekela R**, **McCormack MC**, **Steenbruggen I**, **Stanojevic S**. A Race-neutral Approach to the Interpretation of Lung Function Measurements. *Am J Respir Crit Care Med* 207: 768–774, 2023. doi: 10.1164/rccm.202205-0963OC.

287. **Hussain SN**, **Magder S**. Diaphragmatic intramuscular pressure in relation to tension, shortening, and blood flow. *J Appl Physiol (1985)* 71: 159–167, 1991. doi: 10.1152/jappl.1991.71.1.159.

288. **Hamilton AL**, **Killian KJ**, **Summers E**, **Jones NL**. Muscle strength, symptom intensity, and exercise capacity in patients with cardiorespiratory disorders. *Am J Respir Crit Care Med* 152: 2021–2031, 1995. doi: 10.1164/ajrccm.152.6.8520771.

289. **Baydur A**, **Behrakis PK**, **Zin WA**, **Jaeger M**, **Milic-Emili J**. A simple method for assessing the validity of the esophageal balloon technique. *Am Rev Respir Dis* 126: 788–791, 1982. doi: 10.1164/arrd.1982.126.5.788.

290. **Bellemare F**, **Wight D**, **Lavigne CM**, **Grassino A**. Effect of tension and timing of contraction on the blood flow of the diaphragm. *J Appl Physiol Respir Environ Exerc Physiol* 54: 1597–1606, 1983. doi: 10.1152/jappl.1983.54.6.1597.

291. **Malas FÜ**, **Köseoğlu F**, **Kara M**, **Ece H**, **Aytekin M**, **Öztürk GT**, **Özçakar L**, **Ulaşlı AM**. Diaphragm ultrasonography and pulmonary function tests in patients with spinal cord injury. *Spinal Cord* 57: 679–683, 2019. doi: 10.1038/s41393-019-0275-3.

292. **Barnes WS**. The relationship between maximum isometric strength and intramuscular circulatory occlusion. *Ergonomics* 23: 351–357, 1980. doi: 10.1080/00140138008924748.

293. **Parmar HR**, **Sears J**, **Molgat-Seon Y**, **McCulloch CL**, **McCracken LA**, **Brown CV**, **Sheel AW**, **Dominelli PB**. Oral contraceptives modulate the muscle metaboreflex in healthy young women. *Appl Physiol Nutr Metab* 43: 460–466, 2018. doi: 10.1139/apnm-2017-0482.

294. **Edwards RH**. Human muscle function and fatigue. *Ciba Found Symp* 82: 1–18, 1981. doi: 10.1002/9780470715420.ch1.

295. **Amann M**, **Calbet JAL**. Convective oxygen transport and fatigue. *J Appl Physiol (1985)* 104: 861–870, 2008. doi: 10.1152/japplphysiol.01008.2007.

296. **Lott MEJ**, **Hogeman C**, **Herr M**, **Bhagat M**, **Sinoway LI**. Sex differences in limb vasoconstriction responses to increases in transmural pressures. *Am J Physiol Heart Circ Physiol* 296: H186–H194, 2009. doi: 10.1152/ajpheart.00248.2008.

297. **Murphy WG**. The sex difference in haemoglobin levels in adults - mechanisms, causes, and consequences. *Blood Rev* 28: 41–47, 2014. doi: 10.1016/j.blre.2013.12.003.

298. **Machuki JO**, **Zhang HY**, **Harding SE**, **Sun H**. Molecular pathways of oestrogen receptors and β-adrenergic receptors in cardiac cells: Recognition of their similarities, interactions and therapeutic value. *Acta Physiol (Oxf)* 222: e12978, 2018. doi: 10.1111/apha.12978.

299. **Shoemaker JK**, **Halliwill JR**, **Hughson RL**, **Joyner MJ**. Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery. *American Journal of Physiology-Heart and Circulatory Physiology* 273: H2388–H2395, 1997. doi: 10.1152/ajpheart.1997.273.5.H2388.

300. **Supinski GS**, **Bark H**, **Guanciale A**, **Kelsen SG**. Effect of alterations in muscle fiber length on diaphragm blood flow. *J Appl Physiol (1985)* 60: 1789–1796, 1986. doi: 10.1152/jappl.1986.60.5.1789.

301. **McClatchey PM**, **Williams IM**, **Xu Z**, **Mignemi NA**, **Hughey CC**, **McGuinness OP**, **Beckman JA**, **Wasserman DH**, **Poole DC**, **Akerstrom T**, **Goldman D**, **Fraser GM**, **Ellis CG**. Reply to Letter to the Editor: Perfusion controls muscle glucose uptake by altering the rate of glucose dispersion in vivo. *Am J Physiol Endocrinol Metab* 318: E313–E317, 2020. doi: 10.1152/ajpendo.00508.2019.

302. **Poole DC**, **Pittman RN**, **Musch TI**, **Østergaard L**. August Krogh's theory of muscle microvascular control and oxygen delivery: a paradigm shift based on new data. *J Physiol* 598: 4473–4507, 2020. doi: 10.1113/JP279223.

303. **Poole DC**, **Copp SW**, **Ferguson SK**, **Musch TI**. Skeletal muscle capillary function: contemporary observations and novel hypotheses. *Exp Physiol* 98: 1645–1658, 2013. doi: 10.1113/expphysiol.2013.073874.

304. **Hawkins WW**, **Speck E**, **Leonard VG**. Variation of the Hemoglobin Level with Age and Sex. *Blood* 9: 999–1007, 1954. doi: 10.1182/blood.V9.10.999.999.

305. **Ozemek C**, **Bonikowske A**, **Christle J**, **Gallo P**. *ACSM's Guidelines for Exercise Testing and Prescription*. Lippincott Williams & Wilkins, 2025.

306. **Leenen FH**, **Boer P**, **Dorhout Mees EJ**. Antihypertensive effect of propranolol at rest and during exercise. *Br J Clin Pharmacol* 15: 361–365, 1983. doi: 10.1111/j.1365-2125.1983.tb01511.x.

307. **Wong GW**, **Wright JM**. Blood pressure lowering efficacy of nonselective beta-blockers for primary hypertension. *Cochrane Database Syst Rev* 2014: CD007452, 2014. doi: 10.1002/14651858.CD007452.pub2.

308. **Hart EC**, **Charkoudian N**, **Wallin BG**, **Curry TB**, **Eisenach JH**, **Joyner MJ**. Sex differences in sympathetic neural-hemodynamic balance: implications for human blood

pressure regulation. *Hypertension* 53: 571–576, 2009. doi: 10.1161/HYPERTENSIONAHA.108.126391.

309. **Doherty CJ**, **Thompson BP**, **Rynne PJ**, **Chang J-C**, **Angus SA**, **Aguero AJ**, **Gartner SL**, **Dominelli P**. Exercise-induced bronchodilation is not impacted by beta-adrenergic receptor blockade in males or females. .

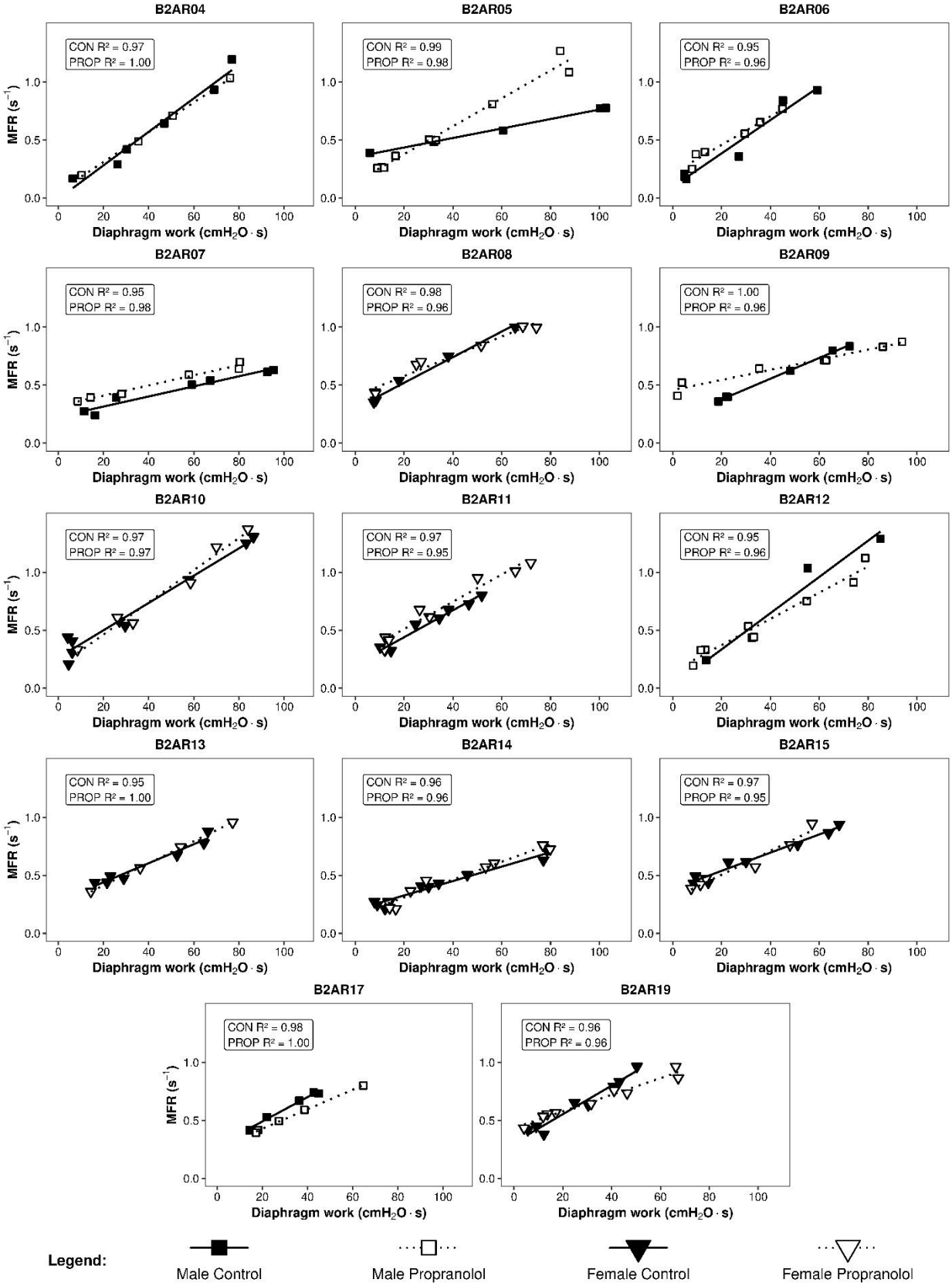
Appendix

Appendix A : Individual diaphragm perfusion vs respiratory pressure responses

Individual participant responses for diaphragm microvascular flux rate (MFR), diaphragm blood flow ($\dot{Q}_{DIA}$), diaphragm vascular conductance (DVC) derived from MFR (DVC_{MFR}), and DVC derived from $\dot{Q}_{DIA}$ ($DVC_{\dot{Q}DIA}$) versus diaphragm work and transdiaphragmatic pressure (P_{DIA}) relative to max. These data demonstrate the variability in diaphragm perfusion and respiratory pressures between conditions.

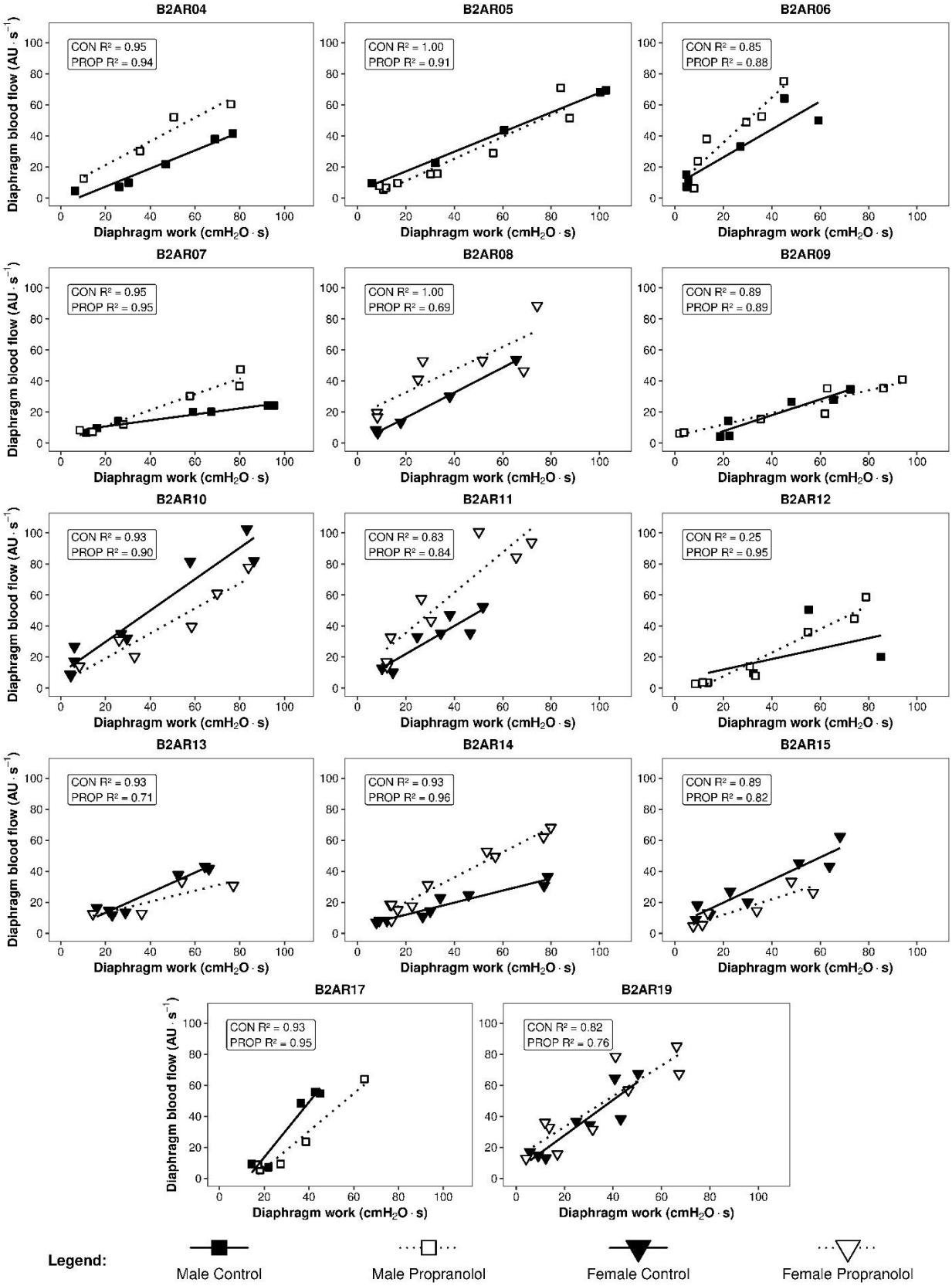
A-1

Diaphragm flux rate vs. Diaphragm work

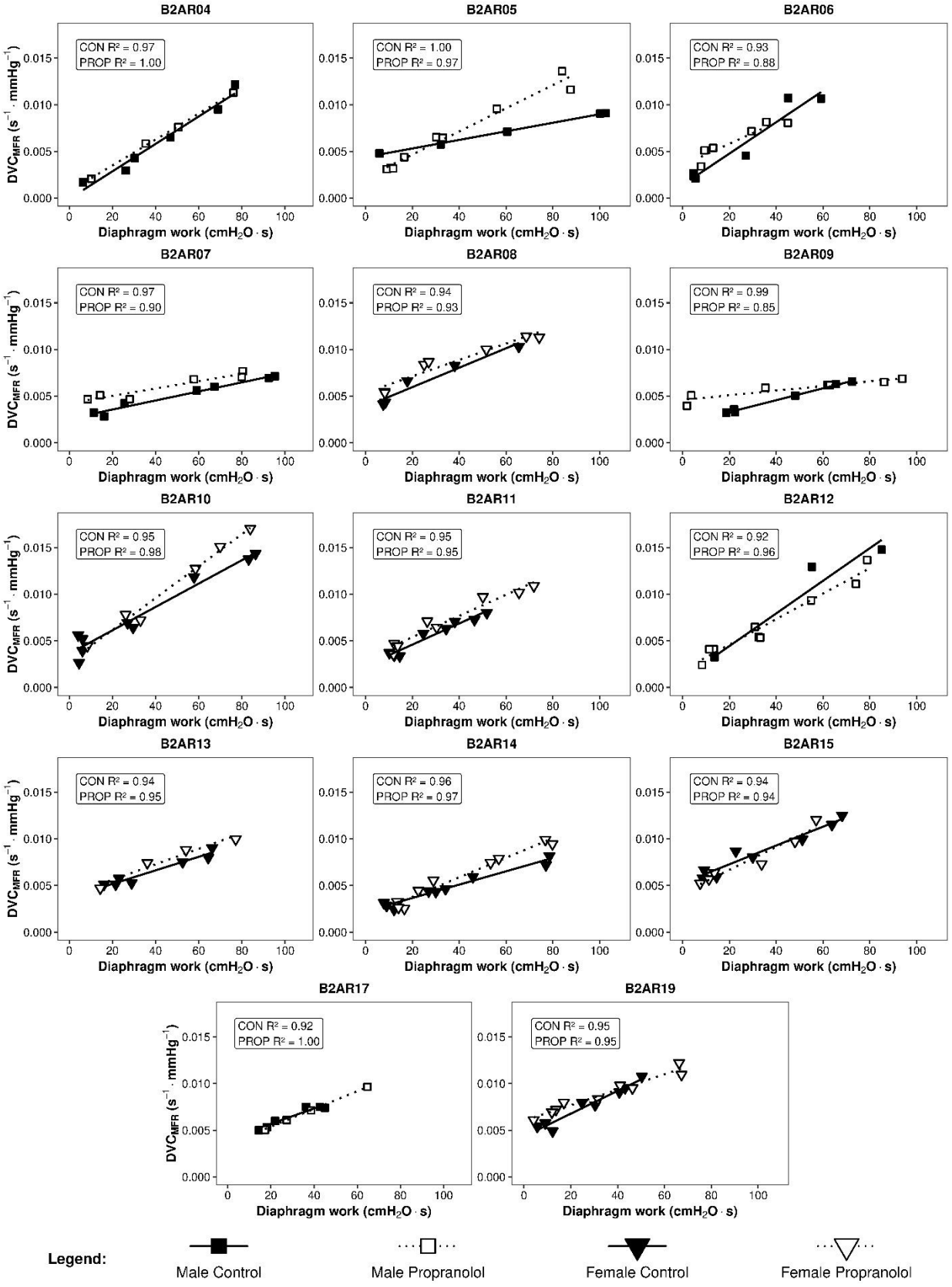


A-2

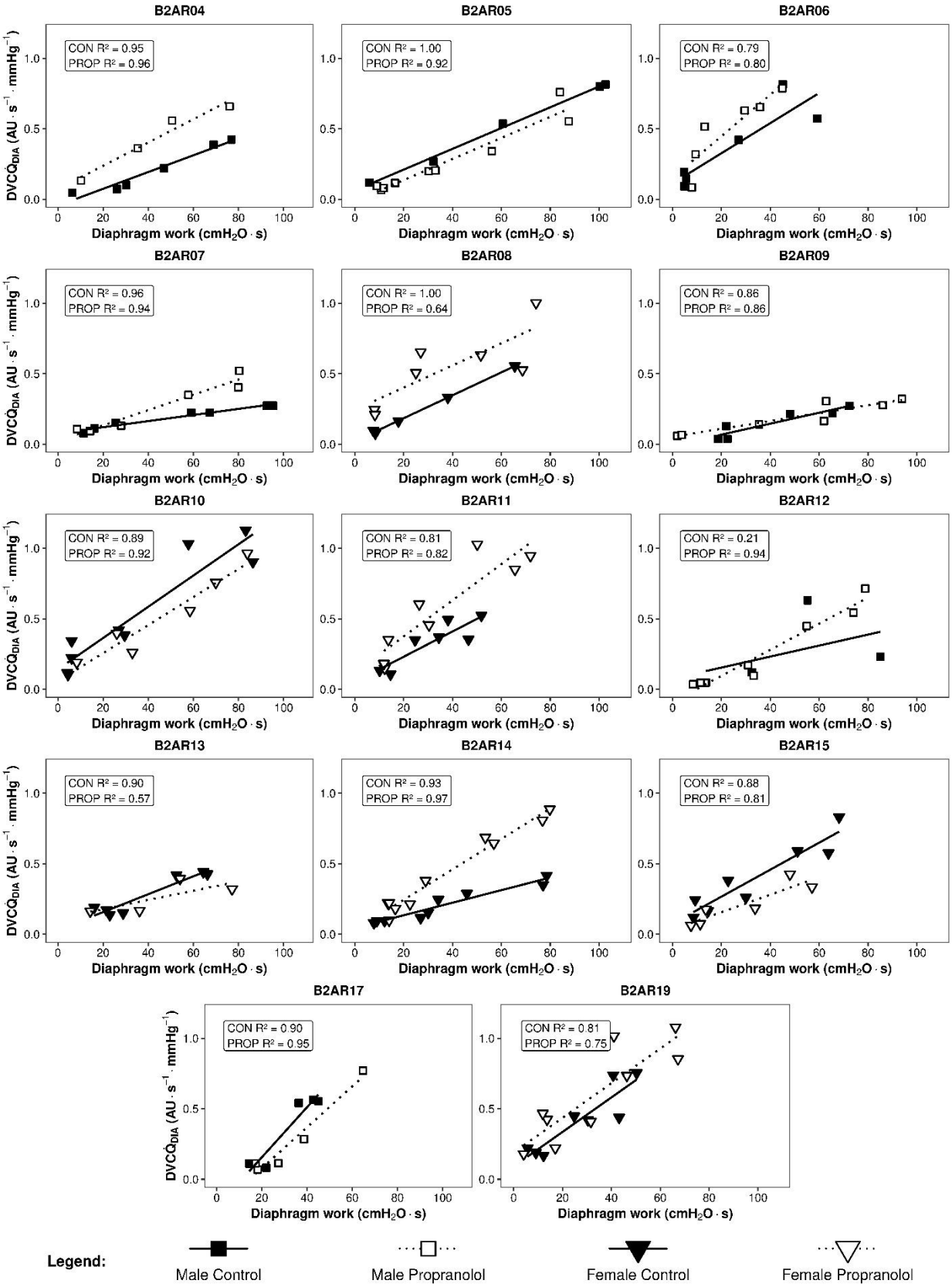
Diaphragm blood flow vs. Diaphragm work



A-3 Diaphragm vascular conductance (MFR) vs. Diaphragm work

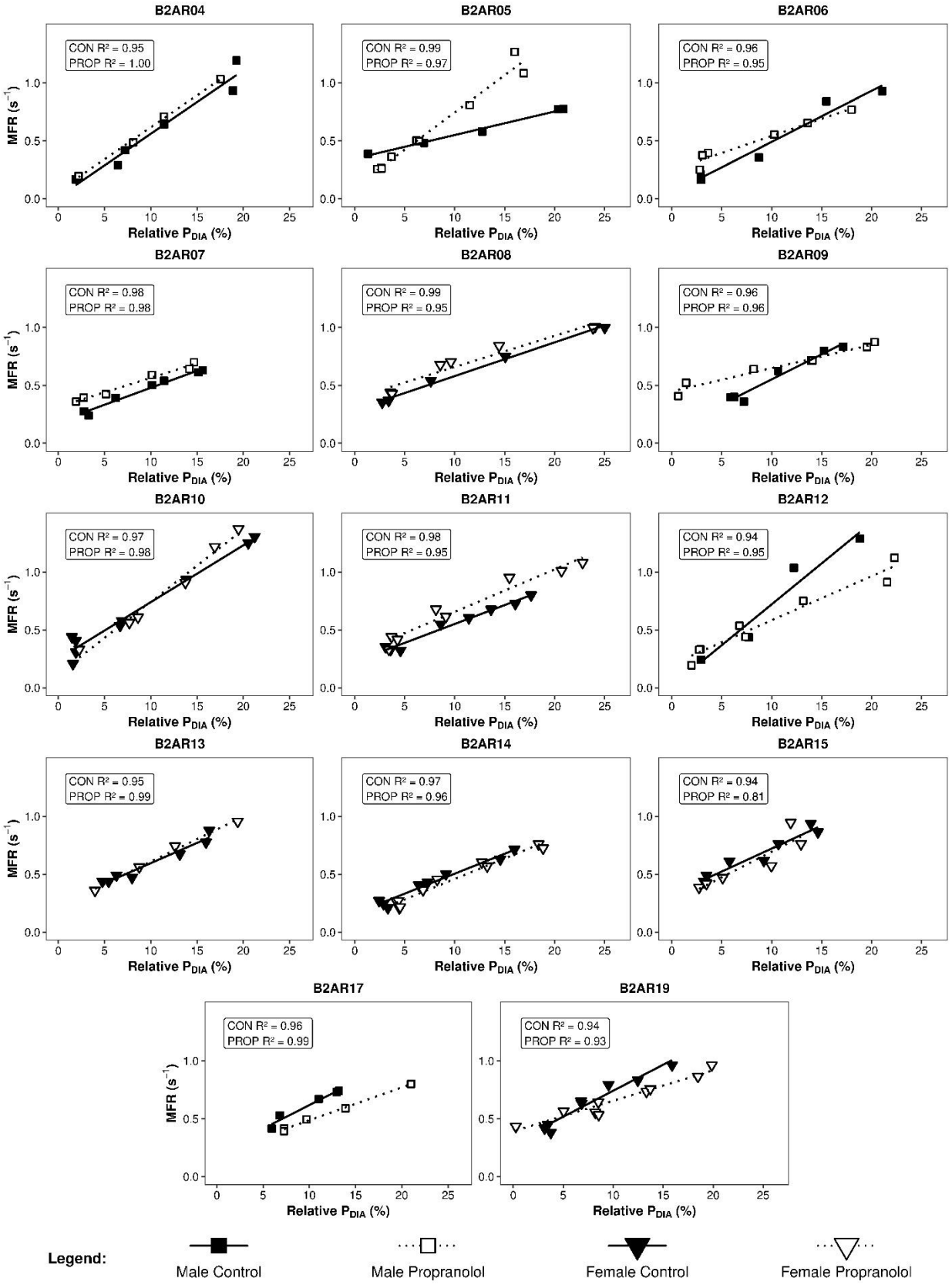


A-4 Diaphragm vascular conductance ($\dot{Q}_{DIA}$) vs. Diaphragm work



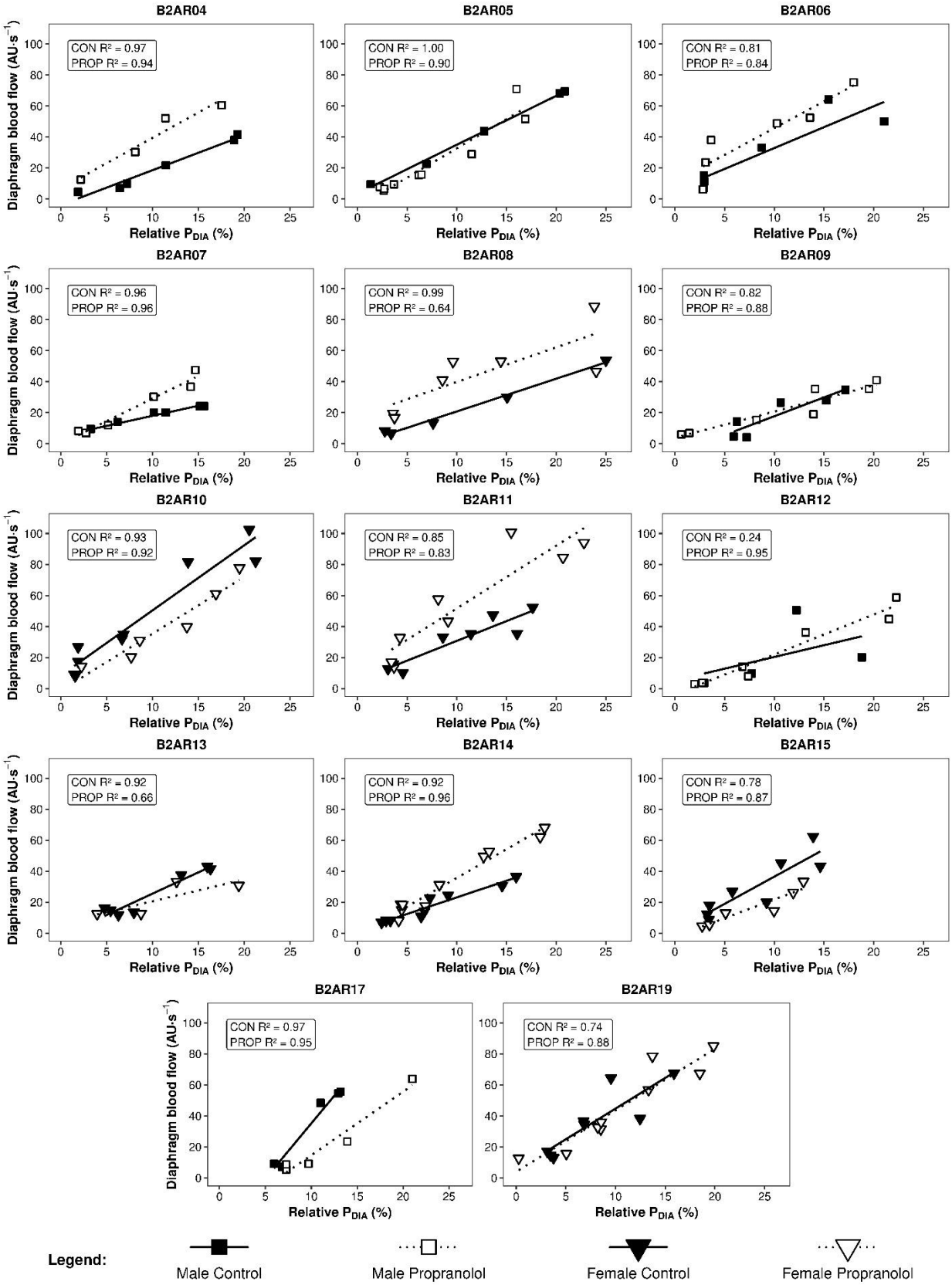
A-5

Diaphragm blood flux rate vs. Relative P_{DIA}

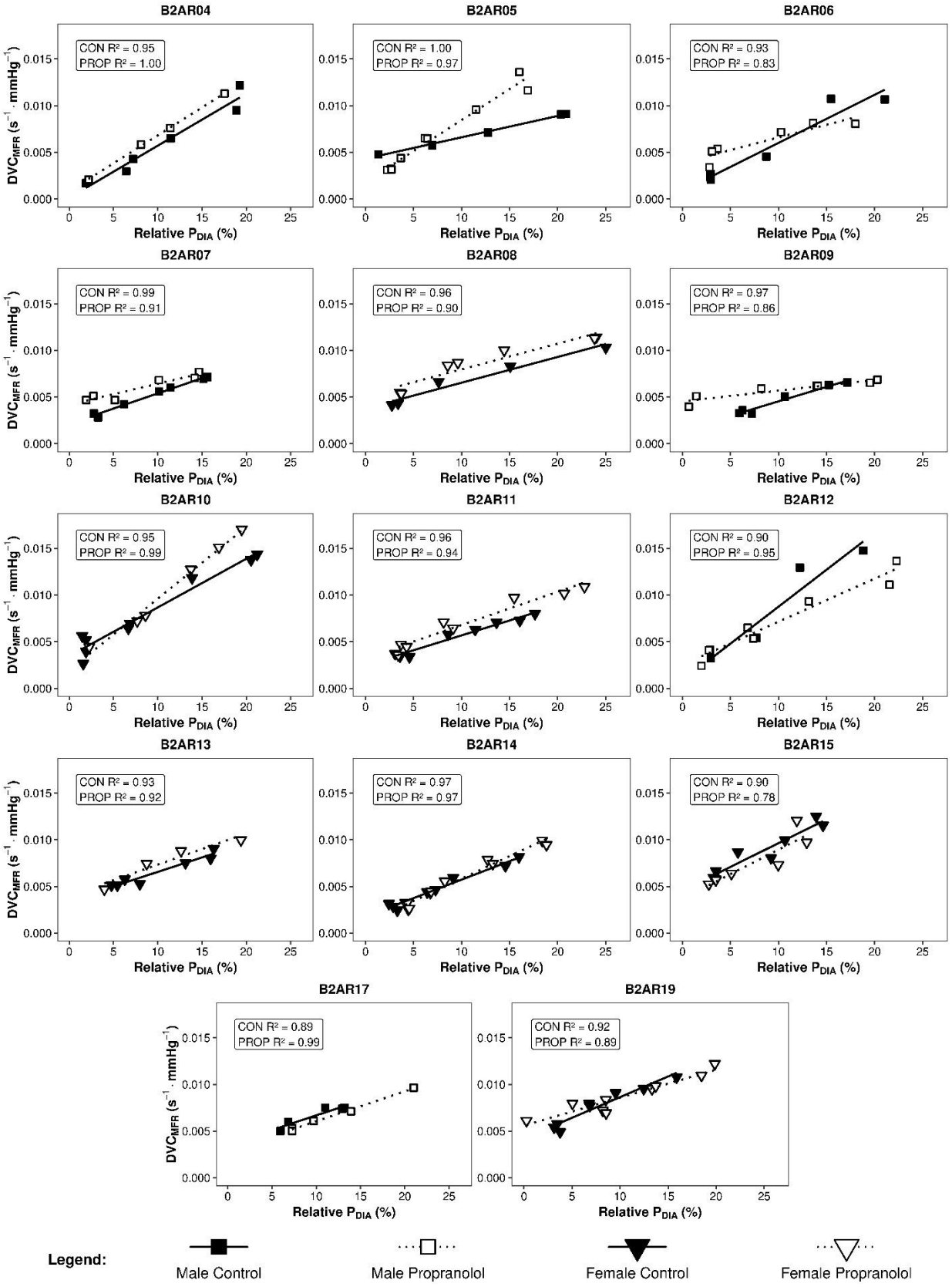


A-6

Diaphragm blood flow vs. Relative P_{DIA}



A-7 Diaphragm vascular conductance (MFR) vs. Relative P_{DIA}



A-8

Diaphragm vascular conductance ($\dot{Q}_{DIA}$) vs. Relative P_{DIA}

