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Diaphragmatic blood flow and energy expenditure in the dog. Effects of inspiratory airflow resistance and hypercapnia.

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Research Article

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Diaphragmatic Blood Flow and Energy Expenditure in the Dog

EFFECTS OF INSPIRATORY AIRFLOW RESISTANCE AND HYPERCAPNIA

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ABSTRACT To investigate the mechanisms which enable the diaphragm to preserve ventilation when the work of breathing is elevated, we measured diaphragmatic blood flow ($\dot{Q}$ di) and oxygen consumption ($\dot{V}_{O_2}$ di) in lightly anesthetized dogs. The animals were studied when they breathed quietly, when they inhaled 5% CO_2 in 21% or 14% O_2, or when they inhaled these gas mixtures through moderate to severe inspiratory resistances. $\dot{Q}$ di was determined from the integrated diaphragmatic arteriovenous difference of krypton-85, by the Kety-Schmidt technique. $\dot{V}_{O_2}$ di was calculated as the product of $\dot{Q}$ di and the diaphragmatic arteriovenous oxygen difference ([A-V]O_2 di). Alteration in these parameters consequent to augmentation of ventilatory effort were compared with concomitant alterations in diaphragmatic electrical activity (EMG di) and an inspiratory pleural pressure-time index (PPTI).

Addition of inspiratory resistances combined with inhalation of CO_2 usually increased $\dot{Q}$ di and consistently increased $\dot{V}_{O_2}$ di, EMG di, and PPTI, the maximum increases being approximately 400–1,600% above control levels. In individual animals, as inspiratory resistance was increased, $\dot{V}_{O_2}$ di, EMG di, and PPTI rose in direct proportion to each other.

In the group as a whole, during resistance breathing the oxygen requirements of the diaphragm were met by a combination of increased [A-V]O_2 di and $\dot{Q}$ di. Unlike other skeletal muscles, oxygen extraction tended to plateau at peak loads, whereas blood flow continued to rise as PPTI and $\dot{V}_{O_2}$ di increased. We conclude that augmentation of perfusion permits the diaphragm to sustain high levels of contractile effort when the work of breathing is increased.

INTRODUCTION

Preservation of respiratory gas exchange in diseases which increase the work of breathing depends on the ability of the respiratory muscles to sustain high levels of effort for prolonged periods. The determinants of respiratory muscle endurance under these circumstances are not known. When limb skeletal muscles perform rhythmic work, their ability to sustain effort appears to depend on muscle blood flow, since at very high work rates blood flow fails to increase in proportion to energy expenditure and fatigue ensues (1–4). Tenney and Reese have postulated that these same considerations hold for the respiratory muscles (5), but no data are available to test this hypothesis.

Recently methods have been devised to measure diaphragmatic blood flow ($\dot{Q}$ di)[1] and oxygen consumption ($\dot{V}_{O_2}$ di) in the dog (6). Quantification of these parameters during intense inspiratory efforts would provide valuable insight into the mechanisms by which the diaphragm sustains respiration in the face of increased work loads. The purposes of this report are to describe the alterations in $\dot{Q}$ di, oxygen extraction, and $\dot{V}_{O_2}$ di consequent to graded increases of inspiratory airflow resistance, and to correlate these alterations with the electrical and mechanical manifestations of diaphragmatic contraction. In addition, the increase in diaphragmatic energy expenditure resulting from stimulation of the respiratory chemoreceptors will be assessed.

METHODS

Animal preparation. These studies were performed in 21 adult mongrel dogs which weighed 19–37 kg. The animals were initially anesthetized with pentobarbital, 25 mg/kg intravenously. They were then intubated with a cuffed oroendotracheal tube. After completion of the surgical procedures, the level of anesthesia was maintained for the remainder of

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[1] *Abbreviations used in this paper:* [A-V]O_2 di, oxygen extraction (diaphragmatic arteriovenous oxygen difference); EMG, di diaphragmatic electrical activity; HV, hyperventilation without added resistance; Pa_{CO_2}, Pa_{O_2}, arterial blood carbon dioxide and oxygen pressure, respectively; PPTI, pleural pressure-time index; QB, quiet breathing; $\dot{Q}$ di, diaphragmatic blood flow; R, inspiratory resistance (R_1 and R_2, moderate; R_3, severe); $\dot{V}E$, minute ventilation; $\dot{V}_{O_2}$ di, diaphragmatic oxygen consumption.

TABLE I

The Effects of Quiet Breathing (QB), CO_2 Inhalation without Added Resistance (HV), and CO_2 Inhalation with an Inspiratory Resistance (R) on $\dot{V}_E$, Respiratory Frequency (f), and Arterial Blood Gas Composition (pH, Pa_{CO_2}, Pa_{O_2})

Dog	Wt	Run	Inspired gas		VE	f	pH	PaCO2	PaO2
			% O2	% CO2					
	kg				*liter/min*	*breath/min*		*mm Hg*	
39	23.6	QB	21	0	4.1	9	7.36	40	80
		R_1	21	5	10.1	10	7.24	48	97
40	20.5	QB	21	0	5.5	18	7.35	37	81
		R_1	21	5	9.2	15	7.28	46	94
41	23.2	QB	21	0	4.3	24	7.45	34	90
		R_1	14	6	11.6	20	7.34	46	71
42	23.2	QB	21	0	6.4	16	7.35	39	92
		R_1	14	6	12.2	17	7.26	52	64
43	27.3	QB	21	0	6.4	10	7.43	25	70
		R_1	14	6	13.7	20	7.24	45	60
44	17.7	QB	21	0	2.6	8	7.25	47	81
		R_1	14	6	7.9	22	7.20	53	69
45	20.5	QB	21	0	5.1	20	7.31	45	79
		R_1	14	6	9.9	34	7.22	55	61
46	19.1	QB	21	0	8.5	33	7.35	32	70
		R_1	14	6	15.1	36	7.21	49	63
50	19.8	QB	21	0	4.0	13	7.30	37	77
		R_1	14	6	9.7	22	7.20	50	61
		R_3	21	0	4.2	32	7.16	56	60
51	36.8	QB	21	0	8.4	22	7.46	31	72
		R_1	14	6	18.4	39	7.35	44	57
		R_3	21	0	7.7	44	7.32	55	50
52	24.6	QB	21	0	4.7	20	7.33	44	71
		R_1	14	6	11.0	39	7.24	57	59
54	24.1	QB	21	0	3.1	7	7.27	48	84
		R_1	14	6	10.4	11	7.18	57	67
		R_2	21	0	2.6	8	7.19	52	69
55	32.7	QB	21	0	10.6	19	7.29	42	66
		HV	14	6	46.7	26	7.29	48	57
		R_1	21	5	14.2	27	7.20	56	73
56	27.3	QB	21	0	8.8	21	7.35	39	83
		HV	14	6	42.3	25	7.29	46	74
		R_1	21	5	13.1	21	7.25	49	97
57	26.8	QB	21	0	3.0	11	7.31	43	66
		HV	14	6	11.4	21	7.26	54	67
		R_2	21	5	6.2	15	7.25	56	63
58	26.4	QB	21	0	4.0	12	7.38	35	66
		HV	14	6	12.7	17	7.35	41	68
		R_2	95	5	7.7	18	7.34	46	380

TABLE I—(*Continued*)

Dog	Wt	Run	Inspired gas % O_2	Inspired gas % Co_2	VE	f	pH	$PaCo_2$	PaO_2
	kg				*liter/min*	*breath/min*		*mm Hg*	
59	30.5	QB	21	0	4.6	9	7.30	38	79
		HV	14	6	11.4	16	7.23	51	71
		R_1	14	6	7.6	16	7.20	55	65
		R_3	21	0	3.6	14	7.23	52	65
60	24.5	QB	21	0	4.4	9	7.34	33	77
		HV	14	6	14.0	23	7.25	45	76
		R_1	14	6	9.8	25	7.22	45	72
		R_2	21	5	8.2	33	7.19	46	82
61	25.0	QB	21	0	6.0	30	7.38	33	68
		HV	14	6	15.4	48	7.27	45	67
		R_1	14	6	9.3	48	7.24	50	57
		R_2	21	5	5.9	44	7.15	67	55
62	26.4	QB	21	0	7.8	21	7.42	29	75
		HV	14	6	19.2	32	7.37	41	66
		R_1	14	6	10.4	41	7.23	54	52
73	22.7	QB	21	0	5.4	18	7.30	38	73
		R_3	21	0	7.1	46	7.21	58	68
		R_3	21	0	7.1	45	7.16	64	59

R_1 and R_2 are moderate resistances; R_3 is a severe resistance. The number and weight of each dog are listed in the two left columns.

the study with an intravenous drip of thiamylal sodium, adjusted so that the inner corneal reflex was maintained. The animals breathed spontaneously throughout the study.

A 6F catheter was advanced via the left femoral vein to the lower inferior vena cava to infuse the maintenance anesthetic. A 7F Cournand catheter was introduced into the right femoral artery and advanced retrograde under fluoroscopic control until the tip lay in the left ventricular cavity. This catheter was utilized for the infusion of radioactive krypton. The left femoral artery was cannulated to permit sampling of systemic arterial blood.

$\dot{Q}$ di and $\dot{V}O_2$ di. Diaphragmatic blood flow was measured by a modification of the Kety-Schmidt technique (6). To sample diaphragmatic venous blood, a 7F Hanafee Nash catheter, 80 cm long, was introduced into the right femoral vein and advanced up the inferior vena cava to the level of the diaphragm. With a controlled tip guide wire (Rotoflector, United States Catheter and Instrument Corp., Glens Falls, N. Y.) the catheter tip was directed into the orifice of the left inferior phrenic vein. After the catheter had been advanced several centimeters into the vein, the guide wire was removed. The position of the catheter was assessed at fluoroscopy by injection of 50% Hypaque solution (Winthrop Laboratories, Div. of Sterling Drug, Inc., New York). Prope placement was confirmed at necropsy in all dogs.

Krypton-85 dissolved in saline was infused into the left ventricle at a rate of 250 μCi per min for 20 min. In the last minute of infusion blood was sampled from the femoral artery and left inferior phrenic vein to determine the equilibrium level of tracer in the tissue. Immediately upon cessation of the infusion blood was sampled from both sites at 1- to 2-min intervals for 8–15 min. These samples established the time-concentration curve for the washout of the tracer. During quiet breathing the sampling rate was approximately 5 ml/min. When blood flow increased during resistance breathing the sampling rate was doubled. Previous experience indicated that these rates could be employed without contaminating phrenic venous blood with inferior vena caval blood and that blood sampled from the left inferior phrenic vein is representative of the venous effluent from most of the diaphragm (6). The blood krypton concentration was determined by the method of Hardewig et al. (7).

Blood flow per 100 g of diaphragm ($\dot{Q}$ di) was calculated from the equilibrium tissue concentration and the area (A) between the arterial and inferior phrenic venous tracer washout curves. The tissue tracer level was estimated from the phrenic venous blood krypton concentration (Ceq) and the blood-muscle solubility coefficient λ, taken as 1.0 for krypton-85, with the following equation:

$$\dot{Q}\ \text{di} = 100 \times \lambda \times \text{Ceq/A},$$
$$\text{ml} \times \text{min}^{-1} \times 100\ \text{g}^{-1}. \quad (1)$$

Justification for these assumptions has been discussed in full elsewhere (6).

$\dot{V}_{O_2}$ di was computed as the product of $\dot{Q}$ di and the diaphragmatic arteriovenous oxygen difference $[A\text{-}V]O_2$ di, in volume percent, by using the formula:

$$\dot{V}_{O_2}\ \text{di} = (\dot{Q}\ \text{di} \times [A\text{-}V]O_2\ \text{di})/100,$$
$$\text{ml} \times \text{min}^{-1} \times 100\ \text{g}^{-1}. \quad (2)$$

Blood oxygen content was measured by the method of Van Slyke and Neill (8).

$\dot{V}O_2$ di was converted to absolute values by multiplying the consumption per gram by the weight of the diaphragm. Weight was estimated from the highly significant relationship previously established in the dog (9): diaphragmatic weight in grams = 25.09 + 4.15 × body weight in kilograms.

Diaphragmatic electrical activity (EMG di). EMG di was recorded directly from the left hemidiaphragm in 17 dogs as previously described by us and by others (9, 10). Access to the muscle was provided by a midline laparotomy incision. The electrodes were fashioned from no. 10 fishhooks insulated to within 5 mm of the tip. The stomach was gently retracted and two electrodes were placed approximately 1.5 cm apart, halfway between the central tendon and the anterolateral part of the coastal insertion. The electrical signal was amplified, filtered, and rectified as described by Lourenço and Mueller (11). Both the electromyogram and its integral were recorded on photographic paper (EMG duplex amplifier-integrator, DR 8 recorder, Electronics for Medicine, White Plains, N. Y.). Throughout any given study, the amplification factors were constant.

The reproducibility of EMG di during periods of quiet breathing and augmented respiration throughout any given study suggested that the contact between muscle and electrodes was not significantly affected by the motion of the contracting diaphragm. In addition, electrical activity in any one part of the diaphragm has been shown to be representative of the remainder of the muscle (12). At necropsy the sites of electrode attachment were carefully examined. In each animal electrodes remained firmly attached to the diaphragm and there was no evidence of bleeding at the site of the attachment.

To quantify electrical activity, the height of the integrator step was measured for each breath. These were summed over several minutes in a given study period, and electrical activity for each period was expressed as the average total electrical activity per minute, in arbitrary units of millimeter paper deflection per minute. To compare results among animals, activity measured during quite breathing was designated "control" and values in other periods were expressed as percent of control values.

Pleural pressure-time index (PPTI). The magnitude and duration of the tension developed by the diaphragm during inspiration was assessed from the inspiratory component of pleural pressure in 10 dogs. Pleural pressure was measured through a mushroom catheter which was introduced into the pleural space through a surgical incision in the sixth right intercostal space. The catheter tip was adjusted so that it lay halfway between the sternum and the spine. The surgical incision was closed in layers and any residual pneumothorax was expelled through the catheter by hyperinflating the lungs with a manual breathing bag (Ruben valve, Air Shields Inc., Hatboro, Pa.).

Pleural pressure was recorded for 10 breaths in each study period (Fig. 1). The end-expiratory pressure was taken as base line. The area between this level and the pressure developed during inspiration was measured, and the average area of 5–10 breaths was calculated. In most studies the area under the inspiratory pressure curve was determined by graphic integration, by using the level of pressure at every 0.1-s time line. In two animals the area calculated this way was checked by planimetry; the difference between the two methods was less than 5%. The PPTI was calculated as the product of the area under the inspiratory curve per breath and the respiratory rate. This represents the average pressure developed per minute by the inspiratory muscles; its units are centimeters H_2O.

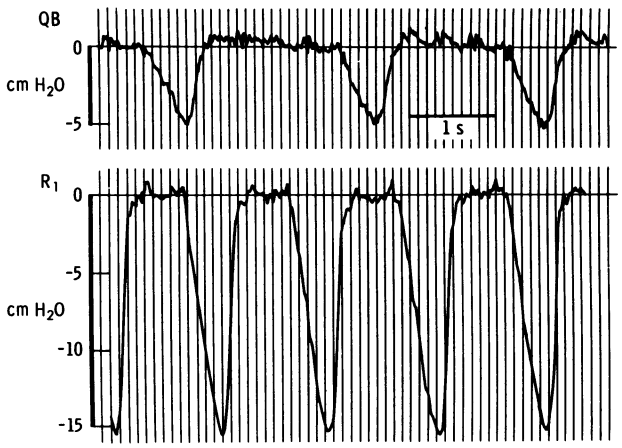

FIGURE 1 Pleural pressure tracing, dog 61. The upper panel was recorded during quiet breathing (QB); the lower panel was recorded while the animal inspired through R_1. In each panel the base line indicates the end-expiratory pressure level, and the downward deflections represent the negative pressure developed during individual inspirations. The PPTI is calculated as the product of the average area between the base line and the inspiratory pressure curve, and the respiratory rate.

Minute ventilation ($\dot{V}_E$), inspiratory resistance, cardiac output. $\dot{V}_E$ was measured by collecting the expired air in a Tissot spirometer. To this end, the endotracheal tube was attached to a low-resistance, low-dead space directional valve (Lloyd valve, Warren E. Collins, Inc., Braintree, Mass.). The expiratory line was connected to the spirometer. The resistances were added to tubing leading to the inspiratory limb of the directional valve.

The resistances used were rubber stoppers with three different orifice sizes (R_1, R_2, R_3). The resistance of each one increased linearly as flow increased. At a constant flow of 0.25 liter/s, the resistances of the endotracheal tube alone and of R_1, R_2, and R_3 were 2, 15, 45, and 190 cm H_2O/(liter/s), respectively. At a flow of 0.5 liter/s, the corresponding values of resistance were 3, 25, 75, and 320 cm H_2O/(liter/s).

Cardiac output was measured in six animals, by using indocyanine green dye (13).

Experimental protocol. $\dot{Q}$ di, $\dot{V}O_2$ di, and EMG di, as well as PPTI, were initially measured during spontaneous quiet breathing to establish control values. Subsequently, these measurements were repeated while the respiratory effort was augmented. This was accomplished in several ways. Eight animals inhaled 6% CO_2 in 14% O_2 without added resistance (Table I, HV). 20 animals inhaled 5 or 6% CO_2 in 14, 21, or 95% O_2 through a moderate inspiratory resistance (Table I, R_1, R_2). Six dogs inhaled 21% O_2, with or without 5% CO_2, through a severe resistance (Table I, R_3). Each intervention was maintained for at least 20 min before measuring $\dot{Q}$ di. $\dot{Q}$ di, [A-V]O_2 di, and $\dot{V}O_2$ di were measured once per run. $\dot{V}_E$, EMG di, and PPTI were measured just before and after the blood flow measurement; the values of these parameters reported for each run represent the average of these two determinations. Ventilation, EMG di, and PPTI were also monitored at frequent intervals between runs.

RESULTS

The values of $\dot{V}_E$, respiratory frequency, and arterial blood gas composition measured in the different study

periods are listed in Table I. Inhalation of carbon dioxide in the absence of an inspiratory resistance increased $\dot{V}_E$ and respiratory frequency (Table I, HV). The combination of carbon dioxide inhalation and a moderate inspiratory resistance had a similar effect on respiratory rate. Under these conditions, $\dot{V}_E$ was always greater than control levels. However, it was lower than when carbon dioxide was inhaled in the absence of the resistance (Table I, R_1 or R_2). The presence of a severe resistance to inspiratory airflow regularly increased the respiratory rate but decreased $\dot{V}_E$ below control levels in three of six animals (Table I, R_3).

As expected, inhalation of carbon dioxide increased arterial blood carbon dioxide tension (Pa_{CO_2}) and decreased pH. Arterial blood oxygen tension (Pa_{O_2}) sometimes rose when the inspired mixture contained 21% O_2, but fell when the inspired mixture contained 14% O_2.

The effects of augmented ventilatory effort on $\dot{Q}$ di, $\dot{V}_{O_2}$ di, and EMG di, as well as on the PPTI developed during inspiration are listed in Table II. $\dot{Q}$ di was 0–67% above control values during hyperventilation without added resistance (period HV), varied from −44 to 157% of control levels when a moderate resistance was employed (periods R_1 or R_2), and increased from 50 to 390% over control levels in the five animals which inspired through the severe resistance (period R_3). The percent change in $\dot{Q}$ di from the control (period QB) to moderate resistance (period R_1 or R_2) was inversely related to the initial Pa_{CO_2}:

$$\Delta\dot{Q}\text{ di} = 289 - 6.29\ Pa_{CO_2} \qquad (3)$$

($n = 20$, $r = -0.70$, $t = 4.13$, $P < 0.001$).

In six animals, cardiac output averaged 172±SD 56 ml/min·kg during quiet breathing and 156±SD 48 ml/min·kg while the animals inspired through the moderate resistance. The difference between periods was not significant ($P > 0.6$). During resistance breathing cardiac output was 3–22% below the control values in five animals and was 26% above the control value in one dog. There was no consistent relationship between cardiac output and $\dot{Q}$ di ($P > 0.7$).

During hyperventilation and resistance breathing, the oxygen requirements of the diaphragm were met by a combination of increased $\dot{Q}$ di and increased $[A\text{-}V]O_2$ di. This is particularly evident when these parameters are plotted as a function of the PPTI, which reflects the force of contraction of the diaphragm (Fig. 2). The relationship between diaphragmatic $\dot{Q}$ di and PPTI in Fig. 2A is linear, according to the formula:

$$\dot{Q}\text{ di} = 12.6 + 5.8\text{PPTI} \qquad (4)$$

($n = 27$, $r = 0.83$, $t = 7.48$, $P < 0.001$). The relationship between $[A\text{-}V]O_2$ di and PPTI (Fig. 2B) could also be fitted with a linear regression. However, in the four animals which achieved the highest levels of PPTI (Table II, dogs 60–62, 73), $\dot{Q}$ di tended to rise progressively with PPTI, whereas $[A\text{-}V]O_2$ di plateaued.

$\dot{V}_{O_2}$ di increased from 57 to 143% over control levels during carbon dioxide induced hyperventilation (period HV), from 40 to 464% while inspiring through the moderate resistance (period R_1 or R_2), and from 110 to 700% when the severe resistance was employed (period R_3). The increases in EMG di during the same periods were 40–248, 44–537, and 136–320%, respectively. Electrical activity was not measured in dogs 51 and 73 which had the greatest increases in $\dot{V}_{O_2}$ di. The PPTI was measured in 10 dogs. It increased from 55 to 200% above control levels during simple hyperventilation in five animals, from 173 to 1,440% during inspiration through a moderate resistance in six animals, and from 375 to 1,580% in the four dogs which experienced the severe resistance.

At the various levels of inspiratory effort, $\dot{V}_{O_2}$ di and EMG di were linearly related in individual animals (Fig. 3a). When these parameters are expressed as a percent change from the control level ([observed − control] × 100/control) the relationship between them for 17 dogs is given by the following equation, which is based on all measurements of EMG di:

$$\Delta\dot{V}_{O_2}\text{ di} = 50.4 + 0.65(\Delta\text{EMG di}) \qquad (5)$$

($n = 31$, $r = 0.66$, $t = 4.75$, $P < 0.001$).

$\dot{V}_{O_2}$ di was also compared with the magnitude and duration of pleural pressure developed during inspiration. As with EMG di, there was a close linear relationship between $\dot{V}_{O_2}$ di and PPTI in individual dogs (Fig. 3B). Moreover, unlike the relationship with EMG di, the correlation between $\dot{V}_{O_2}$ di and PPTI development remained linear when the results from 27 runs in 10 dogs were pooled (Fig. 4).

In dogs 57–62 there were 14 simultaneous measurements of $\dot{V}_{O_2}$ di, EMG di, and PPTI. When these results were expressed as a percent change from the control level, each parameter correlated well with the other two as indicated by the following equations:

$$\Delta\dot{V}_{O_2}\text{ di} = 0.40(\Delta\text{PPTI}) + 53.7 \qquad (6)$$

($n = 14$, $r = 0.88$, $t = 6.37$, $P < 0.001$);

$$\Delta\dot{V}_{O_2}\text{ di} = 0.75(\Delta\text{EMG di}) + 68.3 \qquad (7)$$

($n = 14$, $r = 0.77$, $t = 4.17$, $P < 0.001$);

$$\Delta\text{EMG di} = 0.44(\Delta\text{PPTI}) + 16.8 \qquad (8)$$

($n = 14$, $r = 0.93$, $t = 8.77$, $P < 0.001$).

The response of the diaphragm to carbon dioxide stimulation of the respiratory center was examined both during hyperventilation without added resistance and when the animals inspired through moderate or severe resistances (Table III). When all experiments

TABLE II

The Effects of Quiet Breathing (QB), Augmented Ventilation without Added Resistance (HV), and an Inspiratory Resistance (R) on $\dot{Q}$ di, $[A\text{-}V]O_2$ di and $\dot{V}_{O_2}$ di

Dog	Condition	$\dot{Q}$ di	$[A\text{-}V]O_2$ di	$\dot{V}_{O_2}$ di	EMG di	PPTI
		ml/min·100 g	*vol%*	*ml/min·100 g*	*mm/min*	*cm H_2O*
39	QB	30	4.2	1.2	—	—
	R_1	31	7.0	2.2	—	—
40	QB	23	5.5	1.3	25	—
	R_1	29	8.1	2.3	36	—
41	QB	19	6.7	1.2	92	—
	R_1	26	12.7	3.3	314	—
42	QB	22	4.2	0.9	15	—
	R_1	30	9.9	2.9	44	—
43	QB	18	8.7	1.6	—	—
	R_1	33	13.2	4.3	—	—
44	QB	28	4.5	1.3	58	—
	R_1	32	8.0	2.6	125	—
45	QB	36	2.6	0.9	56	—
	R_1	33	7.0	2.3	184	—
46	QB	30	10.7	3.2	174	—
	R_1	66	11.9	7.9	454	—
50	QB	31	4.3	1.4	84	—
	R_1	28	8.4	2.3	228	—
	R_3	42	10.2	4.3	335	—
51	QB	22	7.7	1.7	—	—
	R_1	48	12.0	5.6	—	—
	R_3	108	12.6	13.6	—	—
52	QB	26	5.6	1.4	125	—
	R_1	40	8.0	3.2	435	—
54	QB	32	1.6	0.5	52	—
	R_1	18	4.9	0.9	133	1.3
	R_2	19	3.7	0.7	98	1.1
55	QB	29	8.3	2.4	69	—
	HV	39	9.9	3.9	141	—
	R_1	54	12.6	6.8	179	4.7
56	QB	18	4.2	0.8	116	—
	HV	25	6.5	1.6	218	—
	R_1	32	8.3	2.6	264	4.6
57	QB	14	4.7	0.7	43	0.6
	HV	19	5.9	1.1	150	—
	R_2	14	8.4	1.2	168	3.0
58	QB	18	7.0	1.2	45	1.1
	HV	22	12.1	2.7	65	1.7
	R_2	21	13.3	2.8	69	3.0
59	QB	10	10.1	1.0	75	0.8
	HV	16	14.8	2.4	152	1.3
	R_1	15	14.2	2.2	177	3.4
	R_3	15	13.7	2.1	177	3.8

TABLE II—(*Continued*)

Dog	Condition	$\dot{Q}$ di	[A-V]O_2 di	$\dot{V}_{O_2}$ di	EMG di	PPTI
		ml/min·100 g	*vol%*	*ml/min·100 g*	*mm/min*	*cm H_2O*
60	QB	20	8.8	1.6	25	1.5
	HV	20	12.9	2.6	35	2.7
	R_1	38	14.4	5.5	55	6.9
	R_3	45	15.8	7.1	80	10.9
61	QB	15	6.0	0.9	120	0.9
	HV	25	7.5	1.9	249	2.4
	R_1	37	10.1	3.7	411	5.0
	R_3	48	9.8	4.7	504	8.0
62	QB	23	6.0	1.4	67	0.6
	HV	32	10.5	3.4	150	1.8
	R_1	59	13.4	7.9	427	6.9
73	QB	13	13.6	1.8	—	0.5
	R_2	48	19.1	9.1	—	7.7
	R_3	56	19.1	10.6	—	8.4

In some animals the responses of EMG di and PPTI to augmented ventilation and inspiratory resistances are also shown.

are considered, the ratio of the increase in $\dot{V}_{O_2}$ di to the increase in Pa_{CO_2} was significantly greater ($P < 0.05$) with either level of resistance than with hyperventilation alone. However, if one examines only those animals, 55 through 62, in which measurements were made during both hyperventilation and resistance breathing, then $\Delta\dot{V}_{O_2}$ di/ΔPa_{CO_2} was not significantly different in the two conditions (Table III). Further, there was no difference in $\Delta\dot{V}_{O_2}$ di/ΔPa_{CO_2} between R_1 and R_3 ($P < 0.2$). There was no correlation between $\Delta\dot{V}_{O_2}$ di/ΔPa_{CO_2} and the control Pa_{CO_2} ($P > 0.3$), but the percent increase in $\dot{V}_{O_2}$ di over control levels was inversely correlated with the control Pa_{CO_2} level:

$$\Delta\dot{V}_{O_2}\text{ di} = 484 - 8.4\ Pa_{CO_2} \quad (9)$$

($n = 20$, $r = -0.53$, $t = 2.67$, $P < 0.02$).

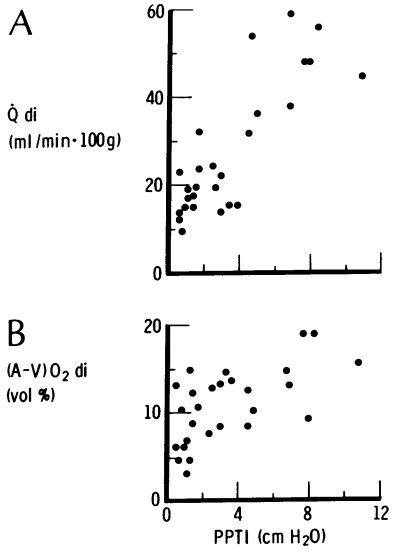

FIGURE 2 The relationships in 10 dogs between $\dot{Q}$di and PPTI in panel A, and between [A-V]O_2 di and PPTI in panel B. In panel A the regression equation is: $\dot{Q}$ di = 12.6 + 5.8PPTI ($n = 27$, $r = 0.83$, $t = 7.48$, $P < 0.001$).

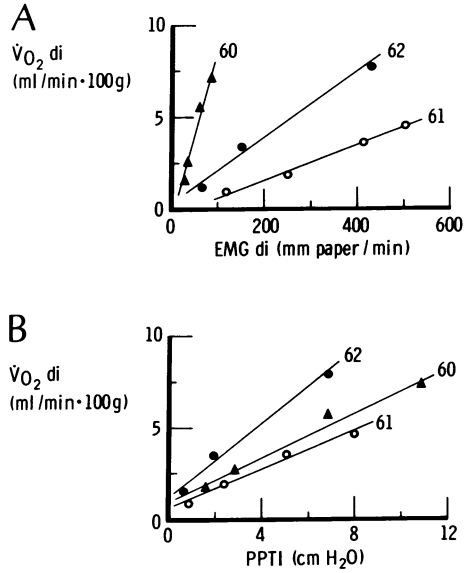

FIGURE 3 $\dot{V}_{O_2}$ di as a function of EMG di in panel A, and of PPTI in panel B. Data from dogs 60–62 are shown in each graph.

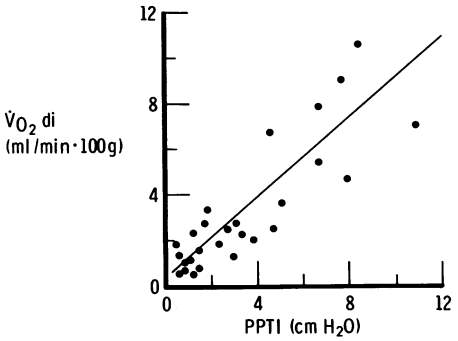

FIGURE 4 $\dot{V}_{O_2}$ di as a function of PPTI in 10 animals. The equation of the regression line is: $\dot{V}_{O_2}$ di = 0.46 + 0.81PPTI (n = 27, r = 0.86, t = 8.33, $P < 0.001$).

DISCUSSION

Q̇ di. The major finding of this study is that Q̇ di generally increased in proportion to the energy requirements of the diaphragm. This result must be considered in light of factors which are known to influence blood flow in the diaphragm and other skeletal muscles.

During quiet breathing, Q̇ di in anesthetized dogs appears to depend on cardiac output (6, 9). In addition, when ventilation is augmented by inhalation of carbon dioxide, it is possible to demonstrate, by multiple regression analysis, that Q̇ di flow is a significant joint function of cardiac output and increased contraction of the diaphragm (9).

In the present study cardiac output was measured in six dogs. In four dogs Q̇ di rose while cardiac output fell, and in one animal both rose together. In another animal, while cardiac output fell from 8 to 67% of the control value, Q̇ di initially fell 10% with R_1 and then increased to 36% above control with R_2. The decrements in cardiac output are consistent with previous experiences in this laboratory with the anesthetized dog whose abdomen is open. No significant relationship between cardiac output and diaphragmatic perfusion during resistance breathing could be demonstrated.

The laparotomy per se does not seem to effect Q̇ di measured by the Kety-Schmidt technique. Measurements were made in nine dogs whose abdomen was intact (6) and in eight whose abdomen had been opened. Neither at quiet breathing nor during hyperventilation were the levels of Q̇ di different in the two groups of animals ($P > 0.1$).

The level of anesthesia may have depressed Q̇ di by lowering cardiac output and $\dot{V}_E$ (14). The finding that Q̇ di during resistance breathing tended to decline or to increase only slightly when the control Pa_{CO_2} was high (Table II, Eq. 3) supports this hypothesis.

Since most dogs were given carbon dioxide while they inhaled through the inspiratory resistance, they developed significant respiratory acidosis (pH 7.15–7.25). Most dogs also developed significant hypoxemia (Pa_{O_2} 50–60 mm Hg) from several sources: inspiration of a low oxygen mixture, alveolar hypoventilation, and intrapulmonary lesions such as atelectasis, pneumonia, or bronchial secretions. Hypercapnic acidosis exerts a weak vasodilating effect on resting skeletal muscle (15, 16), while systemic hypoxemia appears to have no influence (17). We used multiple regression techniques (18, 19) in an attempt to explain the variance of Q̇ di in terms of the force of contraction (PPTI), arterial hydrogen ion concentration, and the degree of arterial

TABLE III

The Ratio of Increase in $\dot{V}_{O_2}$ di to Increase in Pa_{CO_2} in Simple Hyperventilation (HV), Moderate Inspiratory Resistance (R_1 or R_2), and severe inspiratory resistance (P_3)

Dog	$\Delta\dot{V}_{O_2}$ di/$\Delta Paco_2$		
	HV	R_1 or R_2	R_3
30	0.20	—	—
31	0.03	—	—
32	0.08	—	—
33	0.11	—	—
34	0.04	—	—
35	0.04	—	—
38	0.04	—	—
39	—	0.13	—
40	—	0.11	—
41	—	0.18	—
42	—	0.15	—
43	—	0.14	—
44	—	0.22	—
45	—	0.14	—
46	—	0.28	—
50	—	0.07	0.15
51	—	0.30	0.50
52	—	0.14	—
54	—	0.05	—
55	0.25	0.31	—
56	0.11	0.18	—
57	0.04	0.04	—
58	0.25	0.23	—
59	0.11	0.07	0.08
60	0.10	0.33	0.42
61	0.08	0.16	0.11
62	0.17	0.26	—
73	—	—	0.40
Grand Mean	0.12	0.17	0.28
SD	0.07	0.09	0.18
Dogs 55–62 Mean	0.14	0.20	—
SD	0.08	0.11	—

blood oxyhemoglobin unsaturation. Addition of the last two terms failed to reduce the unexplained variance. Hence, only diaphragmatic contractile force appears to have a significant relationship to diaphragmatic perfusion.

Chemical and mechanical factors within contracting muscle are thought to have a profound effect in the hyperemia of exercise. Metabolic and electrolyte changes are important in limb muscles (20, 21), but we have no data concerning the effect of these metabolites in the diaphragm.

Mechanical factors include the effect on muscular arteries and veins of the tissue pressure developed during contraction (22–24) and pinching of nutrient vessels by fascial planes (25). Even though tissue pressure during contraction may greatly exceed systolic arterial blood pressure (24), the pinching effect is thought to have a greater effect on overall mean blood flow. Anrep et al. observed a reduction in left inferior phrenic artery blood flow during diaphragmatic contraction (26), but the relative contributions of tissue pressure and pinching have not been assessed for the diaphragm.

$\dot{V}_{O_2}$ di, EMG di, and tension development. Whenever ventilatory effort was augmented, by carbon dioxide inhalation alone or combined with an inspiratory resistance, $\dot{V}_{O_2}$ di and EMG di and the magnitude of pleural pressure developed during inspiration increased. In individual animals these parameters increased in proportion to each other (Figs. 3A, 3B), and the magnitude of each parameter rose progressively as the animals hyperventilated without resistance, inspired through a moderate resistance, and inspired through a severe resistance. However, for the group as a whole, there was considerable overlap in the degree of increase in $\dot{V}_{O_2}$ di, EMG di, and pleural pressure development during the different study periods.

Previous observations of limb skeletal muscle indicate that force of contraction, integrated electrical activity, and oxygen consumption are directly proportional to each other (27–31). Proportionalities between oxygen consumption, electrical activity, and the force of contraction have also been demonstrated for inspiratory muscles. The increment in total body oxygen consumption during induced hyperventilation or resistance breathing is proportional to EMG di in the rabbit (32) and to inspiratory muscle force in man (33). EMG di is also proportional to transdiaphragmatic pressure in man (34). Hence, the proportionalities found in the present study confirm results of previous investigators.

The coefficients of proportionality among muscle oxygen consumption, electrical activity, and tension development are subject to variance from a variety of sources. Fatigue and reduced oxygen delivery to muscle tend to reduce tension in relation to either oxygen consumption or electrical activity (30, 35, 36). These factors could not be evaluated in the present study.

Mechanical factors, including the length-tension relationship of the diaphragm, the velocity of shortening during contraction, and the radius of curvature of the diaphragm might also have modified the relationships between $\dot{V}_{O_2}$ di, EMG di, and pleural pressure development (27, 34, 37–39). We were unable to identify gross changes in the shape or position of the diaphragm just before contraction in the different study periods. Because we were limited to fluoroscopic observation in a single plane, it is possible that positional changes sufficient to produce significant mechanical effects were overlooked.

Some sources of variance in the data may have resulted from the techniques employed in the study. Determination of both $\dot{Q}$ di and $\dot{V}_{O_2}$ di depend on the quality of the blood samples obtained from the left inferior phrenic vein. When proper catheterization procedure and sampling rate are used, contamination with inferior vena cava blood is not a problem (6). Further, the left inferior phrenic vein does not have significant anastomoses with other venous beds, and its drainage appears to be representative for the bulk of the left hemidiaphragm (6).

The method for recording EMG di probably accounted for the marked variations between animals in the relationship between $\dot{V}_{O_2}$ di and EMG di. Even when both parameters are expressed as a percent change from control value, considerable variation remains (Eq. 5). This most probably results from differences among animals in impedance at the electrode-diaphragm contact, since the range of EMG di recorded during quiet breathing varied 12-fold (Table I). In contrast, there is no evidence for significant variation of electrode impedance in individual animals, since under similar physiologic conditions, similar levels of EMG di were observed.

We used the pleural pressure developed during inspiration as an index of diaphragmatic tension development. This procedure might be questioned on the grounds that pleural pressure is asymmetrically distributed at end-expiration and during inspiration (40–42). To minimize variation among animals, pleural pressure was always sampled from the same site. In addition, with the abdomen open to the atmosphere, all of the change in transdiaphragmatic pressure during contraction should be expressed in the pleural cavity. Thus, the pressure recorded should bear a relatively constant relationship to diaphragmatic tension. However, the nature of the relationship is not susceptible to simple mathematical analysis (37) because of the complex curvature of the diaphragm.

Contraction of the extradiaphragmatic inspiratory muscles might also be considered to affect the rela-

tionship between inspiratory pleural pressure development and $\dot{V}_{O_2}$ di. However, whenever negative inspiratory pressure is developed, whether by the diaphragm alone or in concert with inspiratory intercostal and accessory muscles, the diaphragm must develop sufficient tension to balance the pleural pressure. Therefore, contraction of extradiaphragmatic inspiratory muscles should not affect the relationship between diaphragmatic tension and pleural pressure except indirectly, by stabilizing the thoracic cage. The magnitude of this indirect effect could not be assessed in this study.

The high degree of correlation between $\dot{V}_{O_2}$ di and the PPTI is in keeping with the relationship between myocardial oxygen consumption, ventricular cavitary pressure, and heart rate (43). It is also in agreement with the relationship described by McGregor and Becklake between respiratory muscle oxygen consumption and the force of contraction of the inspiratory muscles (33).

The energy metabolism of the diaphragm should reflect that portion of respiratory center output transmitted by the phrenic nerve, since muscle oxygen consumption and innervation rate are proportional (28). Thus, an increase in carbon dioxide stimulation of the respiratory chemoreceptors should be accompanied by an increase in respiratory muscle oxygen consumption. In each animal $\Delta\dot{V}_{O_2}$ di/$\Delta PaCO_2$ was positive, but there was considerable scatter in the value of the ratio (Table III). Modifiers of the response to carbon dioxide which may have been operative under the conditions of this experiment include variation in level of anesthesia (14), degree of hypoxemia (44, 45), stimulation of lung irritant or J receptors (46), and possibly the magnitude of inspiratory resistance. The relative contributions of and interactions between these modifiers of the carbon dioxide response could be assessed only to a limited extent. The ratio $\Delta\dot{V}_{O_2}$ di/ΔPa_{CO_2} did not correlate with either the control level of Pa_{CO_2} or with the degree of hypoxemia in either control or resistance breathing periods. However, the increase in $\dot{V}_{O_2}$ di induced by the moderate resistance was inversely related to the control Pa_{CO_2} (Eq. 9), which implies a depressant effect of anesthesia. The presence of a resistance seemed to increase $\Delta\dot{V}_{O_2}$ di/ΔPa_{CO_2} slightly for the group as a whole, but not in animals in which measurements were made both in simple hyperventilation and with moderate inspiratory resistance (Table III). These results are consonant with those of previous investigators who showed that the inspiratory muscle work and energy expenditure responses to carbon dioxide were unaffected by airway resistance (47, 48).

Conclusions. The observed changes in $\dot{Q}$ di during resistance breathing provide further insight into the nature of the diaphragm and its ability to sustain contraction at high resistive loads. The oxygen requirements are met by an increase in blood flow and an increase in oxygen extraction. In this regard the diaphragm resembles other skeletal muscles (1). However, at higher levels of tension development, extraction of oxygen by the diaphragm tended to plateau (Fig. 2B), whereas $\dot{Q}$ di continued to rise (Fig. 2A). This behavior is unlike limb skeletal muscle (1, 3). It suggests that the diaphragm resembles the heart in that it depends on perfusion to meet its oxygen needs. The biochemical properties of the diaphragm are also more like the heart than limb skeletal muscle in that its enzymes heavily favor aerobic metabolism (49–52). Thus, both the vascular and biochemical components of the diaphragm are apparently well suited to endurance work. This is undoubtedly an important defense against the development of respiratory failure.

Finally, the proportionalities between $\dot{V}_{O_2}$ di, EMG di and intrathoracic pressure should be of value to the investigation of acute respiratory failure in man. Both EMG di and intrathoracic pressure can be measured in very ill patients, by using intraesophageal electrodes and balloons. Changes in the level of either parameter in response to respiratory center stimulation or to a therapeutic intervention can be considered to reflect a comparable change in diaphragmatic metabolism. For EMG di the alteration can be expressed only in relative terms, but levels of pleural pressure provide an absolute index of diaphragmatic energy expenditure.

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