

1 Cardiovascular effects of intravenous colforsin in normal and acute respiratory acidosis  
2 canine models: a dose–response study

3

4 Short title: Effects of colforsin on cardiovascular function

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6 Takaharu Itami<sup>1\*</sup>¶, Kiwamu Hanazono<sup>1¶</sup>, Norihiko Oyama<sup>1</sup>, Tadashi Sano<sup>2</sup>, Kazuto  
7 Yamashita<sup>1</sup>

8

9 <sup>1</sup>Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido,  
10 Japan.

11 <sup>2</sup>Department of Veterinary Science, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

12

13 \*Corresponding author

14 Takaharu Itami, D.V.M., Ph.D.

15 582, Bunkyo-dai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

16 Tel. +81-11-386-1111

17 Fax. +81-11-388-4129

18 E-mail. [t-itami@rakuno.ac.jp](mailto:t-itami@rakuno.ac.jp) (TI)

19

20 ¶These authors contributed equally to this work.

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22 The role of each author is as follows:

Name	Role
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Takaharu Itami	Conceptualization, data curation, formal analysis, investigation, and original draft preparation
Kiwamu Hanazono	Conceptualization, data curation, formal analysis, investigation, and methodology
Norihiko Oyama	Investigation
Tadashi Sano	Investigation
Kazuto Yamashita	Investigation and supervision

## 24 **Abstract**

25           In acidosis, catecholamines are attenuated and higher doses are often required to  
26 improve cardiovascular function. Colforsin activates adenylate cyclase in cardiomyocytes  
27 without mediating the beta adrenoceptor. In this study, six beagles were administered  
28 either colforsin or dobutamine four times during eucapnia (partial pressure of arterial  
29 carbon dioxide 35-40 mm Hg; normal) and hypercapnia (ibid 90-110 mm Hg; acidosis)  
30 conditions. The latter was induced by carbon dioxide inhalation. Anesthesia was induced  
31 with propofol and maintained with isoflurane. Cardiovascular function was measured by  
32 thermodilution and a Swan-Ganz catheter. Cardiac output, heart rate, and systemic  
33 vascular resistance were determined at baseline and 60 min after 0.3 µg/kg/min (low), 0.6  
34 µg/kg/min (middle), and 1.2 µg/kg/min (high) colforsin administration. The median pH  
35 was 7.38 [range 7.34–7.42] and 7.04 [range 7.01–7.08] at baseline in the Normal and  
36 Acidosis conditions, respectively. Endogenous adrenaline and noradrenaline levels at  
37 baseline were significantly ( $P < 0.05$ ) higher in the Acidosis than in the Normal condition.  
38 Colforsin induced cardiovascular effects similar to those caused by dobutamine.  
39 Colforsin increased cardiac output in the Normal condition (baseline: 198.8 mL/kg/min  
40 [range 119.6–240.9], low: 210.8 mL/kg/min [range 171.9–362.6], middle: 313.8  
41 mL/kg/min [range 231.2–473.2], high: 441.4 mL/kg/min [range 373.9–509.3];  $P < 0.001$ )  
42 and the Acidosis condition (baseline: 285.0 mL/kg/min [range 195.9–355.0], low: 297.4  
43 mL/kg/min [213.3–340.6], middle: 336.3 mL/kg/min [291.3–414.5], high: 366.7  
44 mL/kg/min [339.7–455.7] ml/kg/min;  $P < 0.001$ ). Colforsin significantly increased heart  
45 rate ( $P < 0.05$  in both conditions) and decreased systemic vascular resistance ( $P < 0.05$  in  
46 both conditions) compared to values at baseline. Systemic vascular resistance was lower

47 in the Acidosis than in the Normal condition ( $P < 0.001$ ). Dobutamine increased  
48 pulmonary artery pressure, whereas colforsin did not. Colforsin offsets the effects of  
49 endogenous catecholamines and may not increase cardiac output during hypercapnia.

## 50 **Introduction**

51           Catecholamine beta-1 adrenoceptor is present on myocardial cell membranes.  
52    The catecholamine dobutamine binds to the beta-1 adrenoceptor and activates the cyclic  
53    adenosine monophosphate (cAMP) synthetase adenylate cyclase. The cAMP activates  
54    protein kinase A which phosphorylates the L-type calcium ion- and sarcoplasmic  
55    reticulum calcium ion-releasing channels and increases intracellular calcium ion  
56    concentrations. Dobutamine increases cardiac contractility and heart rate [1]. In contrast,  
57    catecholamine beta-2 adrenoceptor is present on the vascular smooth muscle cell  
58    membrane. Protein kinase A activated as described above phosphorylates myosin light-  
59    chain kinase and inhibits actin and myosin gliding. Dobutamine relaxes vascular smooth  
60    muscle, has both positive inotropic and vasodilator effects (inodilator), and is cardiogenic  
61    and vasodilatory action in dogs [2].

62           Colforsin daropate is a forskolin derivative that directly activates adenylate  
63    cyclase in cardiomyocytes and vascular smooth muscle without mediating the  
64    catecholamine beta adrenoceptor. As with dobutamine, colforsin increases cardiac  
65    contractility and reduces peripheral vascular resistance [3,4]. Colforsin has been tested  
66    on human patients with congestive heart failure and it improved their hemodynamics [5].  
67    When forskolin was first discovered, it was poorly soluble in water and its clinical  
68    application as an injection was limited. Colforsin was prepared as a water-soluble  
69    forskolin derivative and became available in 1999 [5]. However, the efficacy of colforsin  
70    was never compared with that of catecholamines and it was not tested on animals or  
71    humans until now. Catecholamines and phosphodiesterase III inhibitors have been  
72    reported as inodilators in dogs. To the best of our knowledge, however, there have been

73 no reports on the cardiovascular effects of colforsin in dogs.

74 In sepsis and acidosis, myocardial beta-1 adrenoceptor is downregulated.

75 Therefore, catecholamine responses to decreases in cAMP decline. For this reason,

76 cardiac contractility is suppressed in sepsis and acidosis [6]. Unlike adrenaline, colforsin

77 improved cardiac function in rat cardiac resection specimens even under acidosis [7].

78 However, no investigation has been conducted on the effects of colforsin in living

79 organisms under acidosis. We hypothesized that colforsin maintains cardiac contractility

80 in acidotic dogs.

81 The purposes of this study were to: 1) investigate the cardiovascular effects of

82 colforsin in dogs and 2) examine the cardiovascular effects of colforsin in an acute

83 respiratory acidosis model induced by carbon dioxide inhalation.

## 84 **Materials and methods**

85

### 86 **Experimental animals**

87           Six beagles (3 females, 3 males) aged 1–2 y and weighing 9.5–12.5 kg (mean ±  
88 SD: 10.9 ± 1.0 kg) were used in this study. The dogs were judged to be in good health  
89 based on the results of physical examinations, complete blood cell counts, and serum  
90 biochemical analyses. The dogs were owned by the university and maintained according  
91 to the principles of the “Guide for the Care and Use of Laboratory Animals” prepared by  
92 Hokkaido University and approved by the Association for Assessment and Accreditation  
93 of Laboratory Animal Care International (AAALAC). The Animal Care and Use  
94 Committee of Hokkaido University approved the study (No. 14-0156). Food (but not  
95 water) was withheld from the dogs for 12 h before the experiment. Dogs in normal and  
96 acidotic condition were administered colforsin or dobutamine. Each dog was anesthetized  
97 4× at 2-week intervals. This study was performed in a randomized crossover design.

98

### 99 **Experimental preparations**

100           All dogs were fitted with 22-gauge catheters (Surflow; Terumo Co. Ltd., Tokyo,  
101 Japan) in both cephalic veins and administered 6 mg/kg propofol (Propoflo 28; Zoetis Co.  
102 Ltd., Tokyo, Japan) intravenously through a catheter placed in the right cephalic vein.  
103 They were orotracheally intubated and connected to a standard circle anesthesia system  
104 (FO-20A; ACOMA Medical Industry Co. Ltd., Tokyo Japan) and a ventilator (Spiritus;  
105 ACOMA Medical Industry Co. Ltd., Tokyo Japan). All dogs received Ringer’s solution

106 (Fuso Pharmaceutical Industries Ltd., Osaka, Japan) at 5 mL/kg/h and vecuronium (Fuji  
107 Pharma Co. Ltd., Tokyo, Japan) by injection at 0.1 mg/kg. They were then intravenously  
108 infused with 0.1 mg/kg/h vecuronium through a catheter in the right cephalic vein to  
109 prevent reflex respiratory muscle movement. The dogs were mechanically ventilated with  
110 oxygen and received 1.3-1.5% end tidal isoflurane anesthesia. The oxygen flow rate was  
111 2 L/min. They were placed in left lateral recumbency and mechanically ventilated at a  
112 respiratory rate of 12 breaths/min and a 1:2 inspiratory-expiratory ratio with volume  
113 control ventilation (tidal volume = 9–13 mL/kg).

114 A 22-gauge catheter was inserted percutaneously into a left dorsal pedal artery.  
115 Three pressure transducers (DT-NN; Merit Medical Co. Ltd., Tokyo, Japan) were  
116 prepared and calibrated against a mercury manometer at 200 mm Hg, 50 mm Hg, and 20  
117 mm Hg for the mean arterial, pulmonary arterial, and right atrial pressures, respectively.  
118 The right neck region was shaved and aseptically prepared. Approximately 0.5 mL of 2%  
119 lidocaine (xylocaine; Astra-Zeneca, Osaka, Japan) was injected subcutaneously. A 5-Fr,  
120 75-cm Swan-Ganz catheter (132F5; Edwards Lifesciences Co. Ltd., Tokyo, Japan) was  
121 inserted into a jugular vein using a 6-Fr introducer (Medikit Catheter Introducer; Medikit  
122 Co. Ltd., Tokyo, Japan). The distal port of the Swan-Ganz catheter was connected to a  
123 pressure transducer and advanced into the pulmonary artery using the characteristic  
124 pressure changes associated with the right ventricle and pulmonary artery. A transducer  
125 was attached to the arterial catheter to measure mean arterial pressures (MAP; mm Hg).  
126 Transducers were connected to the distal and proximal ports of the Swan-Ganz catheter  
127 to measure mean pulmonary arterial pressure (PAP; mm Hg) at the distal port, pulmonary  
128 arterial occlusion pressure (PAOP; mm Hg) at the distal port, and mean right atrial  
129 pressure (RAP; mm Hg) at the proximal port. All pressure transducers were zeroed at the



130 mid-sternum level. The PAOP was measured after distal balloon inflation on the Swan-  
131 Ganz catheter at the end of expiration.

132 Cardiac output (CO; L/min) was determined by thermodilution. Five milliliters  
133 of normal saline (1-4 °C) was rapidly injected manually into the proximal port of the  
134 Swan-Ganz catheter at the end of expiration. Temperature fluctuations were measured  
135 with a thermosensor placed at the tip of the Swan-Ganz catheter. At each time interval,  
136 three consecutive measurements within 10% of each other were recorded and the average  
137 was recorded as the CO. The thermistor on the Swan-Ganz catheter measured the core  
138 body temperature which was maintained between 37.0–37.5 °C by a forced-air patient-  
139 warming machine (Bair Hugger; 3M Japan Co. Ltd., Tokyo, Japan).

140 After the dogs were instrumented, the normal and acidotic conditions were  
141 adjusted according to the arterial blood gas data. Arterial- and mixed venous blood gases  
142 were measured by collecting 1.0 mL blood from the dorsal pedal- and pulmonary arteries  
143 catheterized to a heparinized syringe. Blood gas measurements (GEM-Premier 3000; IL  
144 Japan Co. Ltd., Tokyo, Japan) were corrected to body temperature. When the  
145 cardiovascular parameters were being measured in the normal condition (Normal), the  
146 arterial partial pressure of carbon dioxide (PaCO<sub>2</sub>) was maintained at ~35–40 mm Hg.  
147 When the cardiovascular parameters were being measured in the acidotic condition  
148 (Acidosis), the PaCO<sub>2</sub> was maintained at ~90–110 mm Hg and the pH was ~7.0.  
149 Exogenous hypercapnia was induced by adding dry gaseous carbon dioxide (CO<sub>2</sub>) to the  
150 inspiratory corrugated tube of the anesthesia circuit.

151

152 **Evaluation of cardiovascular parameters**

153 All dogs were stabilized for 30 min after preparation. Then, baseline  
154 cardiovascular parameters and arterial- and mixed venous blood gases were measured and  
155 recorded as follows: heart rate (HR; beats/min) by electrocardiogram with a lead II, and  
156 MAP, PAP, RAP, and PAOP by a multi-parameter anesthetic monitoring system (RMC-  
157 4000 Cardio Master; Nihon Kohden Corporation, Tokyo, Japan). Cardiac index (CI;  
158 mL/min/kg), stroke volume (SVI; mL/beat/kg), systemic vascular resistance (SVRI;  
159 dynes·sec·cm<sup>-5</sup>/kg), and pulmonary vascular resistance (PVRI; dynes·sec·cm<sup>-5</sup>/kg) were  
160 calculated by inserting values into previously published formulae [8].

161 After the baseline measurements, the dogs were intravenously infused with  
162 colforsin (Adehl; Nihonkayaku Co. Ltd., Tokyo, Japan) or dobutamine (Dobutrex;  
163 Shionogi & Co. Ltd., Osaka, Japan) through a 22-gauge catheter inserted into the left  
164 cephalic vein. Colforsin administration was gradually increased to 1 mL/h (0.3  
165 µg/kg/min), 2 mL/h (0.6 µg/kg/min), and 4 mL/h (1.2 µg/kg/min) every 60 min. Similarly,  
166 dobutamine administration was gradually increased to 1 mL/h (5 µg/kg/min), 2 mL/h (10  
167 µg/kg/min), and 4 mL/h (20 µg/kg/min) every 60 min. Colforsin and dobutamine were  
168 diluted with sterile saline (normal saline; Otsuka Pharmaceutical Factory Inc., Tokyo,  
169 Japan) and administered by infusion pump (TOP-5500; TOP Co. Ltd., Tokyo, Japan). All  
170 cardiopulmonary measurements were repeated every 60 min after each dose was  
171 administered. When the cardiovascular parameters were determined after the final dose,  
172 the arterial- and mixed venous blood gases were measured as described above.

173 After the experiment, all dogs received 0.2 mg/kg subcutaneous meloxicam  
174 (Metacam; Boehringer Ingelheim Co. Ltd., Tokyo, Japan) and 0.01 mg/kg intramuscular  
175 buprenorphine (Lepetan injection; Otsuka Pharmaceutical Factory Inc., Tokyo, Japan) for  
176 analgesia and 25 mg/kg intravenous cefazolin (cefazolin sodium; Nichi-Iko Co. Ltd.,

177 Toyama, Japan) to prevent infection. For the Acidosis condition, carbon dioxide  
178 inhalation was terminated and dog PaCO<sub>2</sub> was maintained at 35–40 mm Hg. They were  
179 administered 0.5 g/kg intravenous mannitol (*D*-mannitol injection; Terumo Co. Ltd.,  
180 Tokyo, Japan) for 30 min to lower intracranial pressure. Colforsin and dobutamine were  
181 washed out for 1 h and the dogs recovered from the anesthesia.

182

### 183 **Biochemical examination**

184 Two milliliters of blood was drawn from the arterial catheter to measure baseline  
185 catecholamine (adrenaline, noradrenaline, and dopamine) concentrations. The blood  
186 samples were immediately centrifuged (1,000 × *g* for 10 min at 4° C) to separate the  
187 plasma, which was then stored at -80 °C until analysis. Catecholamine levels were  
188 determined by an external laboratory (BML Inc., Tokyo, Japan). In addition, 2 mL blood  
189 was drawn from the arterial catheter at baseline and at the end of experiment, the plasma  
190 was isolated from them as described above, and the samples were biochemically analyzed  
191 (DRI-CHEM 7000V; Fujifilm Co. Ltd., Tokyo, Japan) in the laboratory at our facility.

192

### 193 **Statistical analysis**

194 The data were processed using a statistical software (BellCurve for Excel; Social  
195 Survey Research Information Co. Ltd., Tokyo, Japan) and online in R v. 3.5.0. (2018-04-  
196 23). A Wilcoxon signed-rank test was used to compare biochemical measurements  
197 between baseline and the end of the experiment. It was also used to compare  
198 cardiovascular variables at each colforsin and dobutamine dose between the Normal and  
199 Acidosis conditions. A rank transformation version of two-way ANOVA was used to

200 compare the Normal and Acidosis conditions. A post-hoc Steel-Dwass test was used to  
201 compare dose-related effects on cardiovascular parameters. Differences were considered  
202 significant when  $P < 0.05$ .

## 203 **Results**

204

### 205 **Blood gases and biochemical analyses in response to colforsin**

206           The blood gas and biochemical test results at baseline and at the end of the  
207 experiment are shown in Table 1. The median pH at baseline was 7.38 (range 7.34–7.42)  
208 for the Normal condition and 7.04 (7.01–7.08) for the Acidosis condition. In both cases,  
209 the pH was slightly lower by the end of the experiment. The PaCO<sub>2</sub> at baseline was 39.5  
210 mm Hg (range 34.0–41.1 mm Hg) for the Normal condition and 97.8 mm Hg (range 92.0–  
211 100.4 mm Hg) for the Acidosis condition.

212           Blood adrenaline and noradrenaline levels were significantly higher in the  
213 Acidosis than in the Normal condition at baseline ( $P < 0.05$  for both). Plasma glucose  
214 level was significantly ( $P < 0.05$ ) higher in the Acidosis condition than the Normal  
215 condition. In the former, plasma potassium was significantly ( $P < 0.05$ ) higher at the end  
216 of the experiment than it was at baseline. Although the peak plasma potassium was 8.3  
217 mmol/L, no arrhythmia was observed.

218

### 219 **Cardiovascular effects of colforsin**

220           The effects of colforsin on the cardiovascular parameters in the Normal and  
221 Acidosis conditions are shown in Table 2. There was an interaction between the effects  
222 of colforsin and pH on both CI and SVRI. Baseline CI, HR, SVI, PAP, and PAOP were  
223 higher and SVRI was lower in the Acidosis condition than the Normal condition, and the  
224 differences were significant ( $P < 0.05$ ). Relative to the baseline value, the rate of increase

225 in CI in the Acidosis condition was greater than that in the Normal condition (Normal vs.  
226 Acidosis: 6% vs. 4%, 0.3  $\mu\text{g}/\text{kg}/\text{min}$ ; 58% vs. 18%, 0.6  $\mu\text{g}/\text{kg}/\text{min}$ ; 122% vs. 29%, 1.2  
227  $\mu\text{g}/\text{kg}/\text{min}$ ). The SVI, DAP, RAP, SVRI, and PAOP were significantly ( $P < 0.05$ )  
228 different between the Normal and Acidosis conditions.

229 CI and HR significantly ( $P < 0.001$ ) increased in response to colforsin  
230 administration relative to the baseline. In contrast, compared to values at baseline,  
231 colforsin significantly lowered SAP ( $P < 0.05$ ), MAP ( $P < 0.05$ ), SVRI ( $P < 0.001$ ), and  
232 PAOP ( $P < 0.001$ ). The numbers of dogs with mean PAP  $> 20$  mm Hg were one (17%)  
233 at baseline, zero (0%) at 0.3  $\mu\text{g}/\text{kg}/\text{min}$ , three (50%) at 0.6  $\mu\text{g}/\text{kg}/\text{min}$ , and four (67%) at  
234 1.2  $\mu\text{g}/\text{kg}/\text{min}$  in the Acidosis condition.

235 Miosis was observed in three dogs (50%) receiving colforsin and four (67%) in  
236 the Acidosis condition but it disappeared within 6 h after the end of colforsin infusion.  
237 Nausea or vomiting was transiently observed in one dog (17%) in the Normal condition  
238 and two dogs (33%) in the Acidosis condition. All dogs ate and drank within 3 h after the  
239 end of the experiment. No dog presented with complications as a result of the drug  
240 administration according to their blood chemistry and general physical examinations 2  
241 weeks after the end of the experiment.

242

## 243 **Blood gases and biochemical analyses in response to** 244 **dobutamine**

245 The blood gas and biochemical test results at baseline and at the end of the  
246 experiment are shown in Table 3. The pH at baseline was 7.38 (range 7.33–7.41) in the  
247 Normal condition and 6.99 (range 6.96–7.05) in the Acidosis condition. In both cases, the

248 pH had slightly decreased by the end of the experiment. The baseline PaCO<sub>2</sub> was 38.2  
249 mm Hg (range 36.0–42.3 mm Hg) in the Normal condition and 109.0 mm Hg (range  
250 101.0–114.3 mm Hg) in the Acidosis condition. Relative to the baseline, arterial oxygen  
251 delivery (DaO<sub>2</sub>) was significantly increased by dobutamine administration in both  
252 conditions ( $P < 0.05$ ).

253 Blood adrenaline and noradrenaline levels were significantly higher in the  
254 Acidosis condition than the Normal condition at baseline ( $P < 0.05$ ). Plasma glucose was  
255 significantly ( $P < 0.05$ ) higher in the Acidosis condition than the Normal condition. In  
256 the latter case, plasma potassium at the end of the experiment was significantly ( $P < 0.05$ )  
257 higher than it was at baseline.

258

## 259 **Cardiovascular effects of dobutamine**

260 The effects of dobutamine on the cardiovascular parameters in the Normal and  
261 Acidosis conditions are shown in Table 4. There were no interactions between  
262 dobutamine treatment and pH in terms of their effects on the cardiovascular parameters.  
263 The CI, SVI, PAP, and PAOP were higher and the SVRI was lower in the Acidosis  
264 condition than the Normal condition at baseline, and the differences were significant ( $P$   
265  $< 0.05$ ). Relative to the baseline value, the rate of increase in CI in the Acidosis condition  
266 was greater than that in the Normal condition (Normal vs. Acidosis: 46% vs. 44%, 5  
267  $\mu\text{g}/\text{kg}/\text{min}$ ; 129% vs. 66%, 10  $\mu\text{g}/\text{kg}/\text{min}$ ; 157% vs. 82%, 20  $\mu\text{g}/\text{kg}/\text{min}$ ). The CI, HR,  
268 SVI, and PAP significantly increased in response to dobutamine administration ( $P <$   
269 0.001). Dobutamine administration significantly lowered SAP ( $P < 0.01$ ), MAP ( $P <$   
270 0.01), DAP ( $P < 0.001$ ), and SVRI ( $P < 0.001$ ) compared to levels at baseline. The

271 numbers of dogs with mean PAP > 20 mm Hg were one (17%) at 10 µg/kg/min and three  
272 (50%) at 20 µg/kg/min in the Normal condition, two (33%) at baseline, and six (100%) >  
273 5 µg/kg/min in the Acidosis condition.

274           Atrial stasis was observed by the end of the experiment in one acidotic dog  
275 receiving dobutamine. Its plasma potassium was 7.5 mmol/L. After the experiment, its  
276 cardiac rhythm reverted to a normal electrocardiogram waveform. No other arrhythmia  
277 was observed. Miosis was observed in four dogs (67%) receiving dobutamine in the  
278 Acidosis condition. However, the miosis disappeared within 6 h after the end of  
279 dobutamine infusion. Nausea or vomiting was transiently observed in three dogs (50%)  
280 in the Normal condition and two dogs (33%) in the Acidosis condition. All dogs ate and  
281 drank within 3 h after the end of the experiment. No dog presented with complications as  
282 a result of the drug administration according to their blood chemistry and general physical  
283 examinations 2 weeks after the end of the experiment.

284



**Table 1. The effects of colforsin on blood gas examination and blood biochemical test in six anesthetized dogs in eucapnia (Normal) and acute respiratory acidosis (Acidosis) at baseline and at the end of the experiment.**

Variable (Unit)	Condition	Baseline	End of experiment	Reference
Adrenaline (ng/mL)	Normal	0.01 [0.01–0.09]	–	<0.10
	Acidosis	0.14 [0.05–0.37]§	–	
Noradrenaline (ng/mL)	Normal	0.03 [0.01–0.46]	–	0.10–0.50
	Acidosis	0.35 [0.17–0.97]§	–	
Dopamine (ng/mL)	Normal	0.01 [0.01–0.04]	–	<0.03
	Acidosis	0.02 [0.02–0.08]	–	
PCV (%)	Normal	28 [24–37]	26 [21–34]	37–55
	Acidosis	39 [29–47]	35 [25–37]	
pH	Normal	7.38 [7.34–7.42]	7.32 [7.29–7.37]*	7.35–7.45
	Acidosis	7.04 [7.01–7.08]†	6.99 [6.92–7.01]‡	
PaCO <sub>2</sub> (mm Hg)	Normal	40 [34–41]	39 [35–45]	30.8–42.8
	Acidosis	98 [92–100]†	110 [106–125]‡	
PaO <sub>2</sub> (mm Hg)	Normal	559 [501–616]	571 [496–619]	80.9–103.3
	Acidosis	522 [488–538]	519 [387–540]	
HCO <sub>3</sub> <sup>-</sup> (mEq/L)	Normal	22.6 [20.8–25.4]	20.3 [19.6–23.2]	18.8–25.6
	Acidosis	26.1 [24.6–27.7]†	27.0 [23.8–28.0]	
DaO <sub>2</sub> I (mL O <sub>2</sub> /min/kg)	Normal	27.1 [17.9–32.3]	56.0 [42.3–75.0]*	–
	Acidosis	50.3 [37.2–62.6]†	61.2 [50.3–71.2]	
VaO <sub>2</sub> I (mL O <sub>2</sub> /min/kg)	Normal	3.9 [2.5–5.6]	6.5 [4.7–7.4]	–
	Acidosis	4.4 [3.1–5.0]	5.5 [2.8–6.3]	
O <sub>2</sub> ER (%)	Normal	16.4 [12.4–20.0]	11.1 [8.1–12.7]	–
	Acidosis	8.3 [7.0–10.5]†	8.6 [5.5–10.7]	
BE <sub>ecf</sub> (mEq/L)	Normal	-2.2 [-4.5–1.0]	-5.6 [-6.5–3.0]	-4–+4
	Acidosis	-4.5 [-7.0–2.8]	-4.6 [-8.0–3.0]	
Lactate (mmol/L)	Normal	1.4 [1.1–2.2]	0.9 [0.7–1.2]*	<2.0
	Acidosis	0.5 [0.3–1.0]†	0.3 [0.3–0.5]	
Na (mEq/L)	Normal	144 [143–145]	145 [144–148]	135–147
	Acidosis	146 [142–150]	144 [139–148]	
K (mEq/L)	Normal	3.8 [3.2–3.9]	3.5 [3.2–3.8]	3.5–5.0
	Acidosis	3.9 [3.8–4.2]	7.2 [6.2–8.3]‡	
Cl (mEq/L)	Normal	115 [111–116]	117 [109–121]	95–125
	Acidosis	115 [110–118]	115 [113–119]	
Glucose (mg/dL)	Normal	104 [92–117]	108 [89–115]	60–110
	Acidosis	136 [114–188]†	128 [117–159]	
BUN (mg/dL)	Normal	13.2 [9.0–17.5]	13.8 [10.0–20.0]	10–20
	Acidosis	15.5 [12.6–22.0]	19.0 [15.6–25.0]	
Creatinine (mg/dL)	Normal	0.4 [0.3–0.6]	0.4 [0.2–0.5]	0.6–1.2
	Acidosis	0.6 [0.4–0.7]	0.9 [0.6–1.2]	

PCV, packed cell volume; PaCO<sub>2</sub>, arterial partial pressure of carbon dioxide; PaO<sub>2</sub>, arterial partial pressure of oxygen; HCO<sub>3</sub><sup>-</sup>, bicarbonate ion; DaO<sub>2</sub>, oxygen delivery; VaO<sub>2</sub>, oxygen consumption; O<sub>2</sub>ER, oxygen extraction ratio; BE<sub>ecf</sub>, base excess in the extracellular fluid; Na, sodium ion; K, potassium ion; Cl, chloride ion; BUN, blood urea nitrogen. The reference values were shown from individual testing apparatus. § shows significant difference ( $P < 0.05$ ) from baseline in Normal condition by Wilcoxon signed-rank test. \* and † show significant difference ( $P < 0.05$ ) from baseline in Normal condition, respectively, and ‡ shows significant difference ( $P < 0.05$ ) from baseline in Acidosis condition by Steel-Dwass, respectively.

**Table 2. Median [range] values for cardiovascular variables at baseline, 0.3 µg/kg/min, 0.6 µg/kg/min, and 1.2 µg/kg/min dose of intravenous colforsin in six anesthetized dogs in eucapnia (Normal) and acute respiratory acidosis (Acidosis) conditions.**

Variable (Unit)	Condition	Baseline	0.3 µg/kg/min	0.6 µg/kg/min	1.2 µg/kg/min	P-value		
						Condition	Treatment	Interaction
CI (mLmin/kg)	Normal	198.8 [119.6–240.9]	210.8 [171.9–362.6]	313.8 [231.2–473.2] <sup>A</sup>	441.4 [373.9–509.3] <sup>AB</sup>	0.189	<0.001	0.033
	Acidosis	285.0 [195.9–355.0]*	297.4 [213.3–340.6]	336.3 [291.3–414.5]	366.7 [339.7–455.7]* <sup>B</sup>			
HR (beats/min)	Normal	81 [68–116]	99 [76–140]	152 [95–173]	197 [188–209] <sup>ABC</sup>	0.194	<0.001	0.071
	Acidosis	114 [94–133]*	120 [99–131]	129 [107–155]	135 [123–165]*			
SVI (mL/beat/kg)	Normal	1.86 [1.46–2.65]	2.13 [2.03–2.57]	2.37 [1.88–2.63]	2.06 [1.88–2.34]	<0.001	0.106	0.356
	Acidosis	2.51 [1.97–2.67]*	2.59 [2.16–2.75]*	2.64 [2.41–2.74]	2.68 [2.53–2.89]*			
SAP (mm Hg)	Normal	101 [68–114]	101 [82–115]	88 [84–110]	81 [70–93]	0.176	0.014	0.894
	Acidosis	96 [81–98]	87 [85–100]	92 [61–102]	79 [49–101]			
MAP (mm Hg)	Normal	71 [50–84]	74 [56–86]	66 [58–77]	59 [36–66]	0.335	0.033	0.835
	Acidosis	69 [51–77]	67 [55–76]	65 [42–74]	57 [36–76]			
DAP (mm Hg)	Normal	57 [40–67]	60 [40–75]	58 [44–60]	50 [42–64]	<0.001	0.078	0.657
	Acidosis	53 [37–59]	47 [39–55]	49 [31–52]	44 [28–50]			
RAP (mm Hg)	Normal	4 [2–6]	3 [2–5]	2 [2–5]	2 [2–5]	<0.001	0.200	0.298
	Acidosis	4 [2–5]	5 [4–6]	4 [3–5]	4 [2–4]			
SVRI (dynes•sec•cm <sup>-5</sup> /kg)	Normal	287.7 [219.2–326.2]	247.2 [134.0–332.9]	154.5 [93.9–213.3] <sup>A</sup>	100.6 [65.8–123.9] <sup>AB</sup>	<0.001	<0.001	0.003
	Acidosis	140.5 [113.7–248.0]*	132.4 [110.5–238.3]	99.7 [90.8–155.7]	80.3 [67.9–133.0]			
PAP (mm Hg)	Normal	11 [10–12]	11 [8–13]	12 [8–16]	13 [10–18]	<0.001	0.167	0.948
	Acidosis	19 [17–21]*	19 [18–19]*	20 [18–21]*	20 [19–22]*			
PAOP (mm Hg)	Normal	4 [4–6]	4 [3–5]	3 [3–5]	3 [2–6]	<0.001	<0.001	0.454
	Acidosis	11 [9–12]*	10 [6–14]*	9 [7–11]*	9 [7–9]* <sup>A</sup>			
PVRI (dynes•sec•cm <sup>-5</sup> /kg)	Normal	24.6 [16.9–49.9]	19.7 [16.4–37.6]	19.0 [11.5–33.7]	19.8 [8.6–25.8]	0.661	0.811	0.201
	Acidosis	19.2 [12.6–22.4]	20.4 [11.5–34.2]	22.7 [13.2–31.3]	21.4 [14.1–23.7]			

CI, cardiac index; HR, heart rate; SVI, stroke volume index; SAP, systolic arterial pressure; MAP, mean arterial pressure, DAP, diastolic arterial pressure; RAP, right atrial pressure; SVRI, systemic vascular resistance index; PAP, pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; PVRI, pulmonary vascular resistance index. The rank transformation version of two-way ANOVA applies on condition and dobutamine treatment. Superscript A, B, and C show significant difference ( $P < 0.05$ ) from baseline, 0.3 µg/kg/min, and 0.6 µg/kg/min by Steel-Dwass, respectively. \* shows a significant difference ( $P < 0.05$ ) as compared with each dose in Normal condition.

**Table 3. The effects of dobutamine on blood gas examination and blood biochemical test in six anesthetized dogs in eucapnia (Normal) and acute respiratory acidosis (Acidosis) at baseline and at the end of the experiment.**

Variable (Unit)	Condition	Baseline	End of experiment	Reference
Adrenaline (ng/mL)	Normal	0.01 [0.01–0.13]	–	<0.10
	Acidosis	0.26 [0.08–2.08]§	–	
Noradrenaline (ng/mL)	Normal	0.04 [0.02–0.09]	–	0.10–0.50
	Acidosis	0.32 [0.24–0.44]§	–	
Dopamine (ng/mL)	Normal	0.01 [0.01–0.02]	–	<0.03
	Acidosis	0.02 [0.01–0.03]	–	
PCV (%)	Normal	34 [27–39]	35 [33–43]	37–55
	Acidosis	40 [34–49]	45 [40–51]	
pH	Normal	7.38 [7.33–7.41]	7.30 [7.25–7.36]*	7.35–7.45
	Acidosis	6.99 [6.96–7.05]†	6.92 [6.86–6.95]‡	
PaCO <sub>2</sub> (mm Hg)	Normal	38 [36–42]	42 [35–46]	30.8–42.8
	Acidosis	109 [101–114]†	126 [115–146]‡	
PaO <sub>2</sub> (mm Hg)	Normal	539 [495–571]	579 [568–607]*	80.9–103.3
	Acidosis	525 [443–551]	505 [473–544]	
HCO <sub>3</sub> <sup>-</sup> (mEq/L)	Normal	22.8 [21.9–24.6]	20.1 [18–21.8]*	18.8–25.6
	Acidosis	26.6 [24.6–27.9]†	25.9 [24.7–27.9]	
DaO <sub>2</sub> I (mL O <sub>2</sub> /min/kg)	Normal	31.7 [20.6–40.7]	89.9 [67.4–109.6]*	–
	Acidosis	49.1 [44.5–59.7]†	101.1 [83.3–112.4]‡	
VaO <sub>2</sub> I (mL O <sub>2</sub> /min/kg)	Normal	4.0 [2.4–4.3]	7.0 [5.5–7.8]*	–
	Acidosis	4.4 [3.3–6.2]	6.5 [4.2–7.3]	
O <sub>2</sub> ER (%)	Normal	13.5 [11.2–17.0]	7.8 [6.1–9.5]*	–
	Acidosis	8.4 [7.1–11.9]	6.1 [4.6–7.9]	
BE <sub>ecf</sub> (mEq/L)	Normal	-2.0 [-3.2–-1.0]	-7.0 [-8.1–-4.0]	-4–+4
	Acidosis	-5.0 [-7.0–-2.6]	-6.6 [-8.0–-5.2]	
Lactate (mmol/L)	Normal	1.5 [0.6–3.2]	0.3 [0.3–0.5]*	<2.0
	Acidosis	0.6 [0.3–0.9]	0.6 [0.3–1.6]	
Na (mEq/L)	Normal	144 [142–148]	146 [143–148]	135–147
	Acidosis	146 [144–148]	146 [141–148]	
K (mEq/L)	Normal	3.7 [3.3–4.3]	3.5 [3.0–4.8]	3.5–5.0
	Acidosis	3.8 [3.2–4.3]	6.1 [5.0–7.5]‡	
Cl (mEq/L)	Normal	115 [111–119]	116 [115–123]	95–125
	Acidosis	113 [109–118]	113 [111–117]	
Glucose (mg/dL)	Normal	103 [89–137]	94 [87–106]	60–110
	Acidosis	150 [123–200]†	138 [121–243]	
BUN (mg/dL)	Normal	15.0 [12.1–17.4]	13.0 [11.0–15.7]	10–20
	Acidosis	15.0 [11.0–22.0]	18.5 [15.0–25.0]	
Creatinin (mg/dL)	Normal	0.5 [0.4–0.9]	0.35 [0.3–0.5]	0.6–1.2
	Acidosis	0.6 [0.5–0.7]	1.05 [0.6–1.4]	

PCV, packed cell volume; PaCO<sub>2</sub>, arterial partial pressure of carbon dioxide; PaO<sub>2</sub>, arterial partial pressure of oxygen; HCO<sub>3</sub><sup>-</sup>, bicarbonate ion; DaO<sub>2</sub>I, oxygen delivery; VaO<sub>2</sub>I, oxygen consumption; O<sub>2</sub>ER, oxygen extraction ratio; BE<sub>ecf</sub>, base excess in the extracellular fluid; Na, sodium ion; K, potassium ion; Cl, chloride ion; BUN, blood urea nitrogen. The reference values were shown from individual testing apparatus. § shows significant difference ( $P < 0.05$ ) from baseline in Normal condition by Wilcoxon signed-rank test. \* and † show significant difference ( $P < 0.05$ ) from baseline in Normal condition, respectively, and ‡ shows significant difference ( $P < 0.05$ ) from baseline in Acidosis condition by Steel-Dwass, respectively.

**Table 4. Median [range] values for cardiovascular variables at baseline, 5 µg/kg/min, 10 µg/kg/min, and 20 µg/kg/min dose of intravenous dobutamine in six anesthetized dogs in eucapnia (Normal) and acute respiratory acidosis (Acidosis) conditions.**

Variable (Unit)	Condition	Baseline	5 µg/kg/min	10 µg/kg/min	20 µg/kg/min	P-value		
						Condition	Treatment	Interaction
CI (mL/min/kg)	Normal	182.8 [119.0–261.8]	267.4 [136.0–362.2]	418.1 [317.7–471.2] <sup>A</sup>	470.3 [421.5–507.4] <sup>AB</sup>	0.027	<0.001	0.253
	Acidosis	252.9 [199.5–301.6]*	364.5 [268.0–446.5]*	418.9 [330.1–494.1] <sup>A</sup>	459.9 [376.4–566.7] <sup>A</sup>			
HR (beats/min)	Normal	96 [60–136]	103 [60–143]	159 [110–204]	188 [166–201] <sup>AB</sup>	0.740	<0.001	0.106
	Acidosis	109 [84–135]	134 [91–174]*	142 [121–180]	161 [138–192]* <sup>A</sup>			
SVI (mL/beat/kg)	Normal	1.96 [1.71–2.26]	2.49 [2.26–2.97] <sup>A</sup>	2.64 [2.31–3.26] <sup>A</sup>	2.50 [2.27–2.76] <sup>A</sup>	0.001	<0.001	0.857
	Acidosis	2.21 [2.02–2.70]*	2.62 [2.45–3.53]*	2.86 [2.49–3.37]	2.87 [2.46–3.35]			
SAP (mm Hg)	Normal	93 [82–108]	98 [76–116]	94 [65–102]	81 [60–102]	0.628	0.008	0.847
	Acidosis	97 [80–105]	104 [86–112]	83 [62–105]	66 [54–101]			
MAP (mm Hg)	Normal	68 [55–76]	70 [51–83]	68 [52–78]	59 [47–72]	0.652	0.007	0.784
	Acidosis	68 [57–75]	71 [62–89]	61 [47–73]	52 [44–70]			
DAP (mm Hg)	Normal	55 [43–65]	56 [38–62]	50 [40–58]	44 [36–51]	0.295	<0.001	0.780
	Acidosis	51 [43–55]	54 [49–73]	48 [37–54]	40 [374–52]			
RAP (mm Hg)	Normal	3 [2–5]	3 [2–5]	2 [2–5]	2 [2–4]	<0.001	1.000	1.000
	Acidosis	5 [1–6]	5 [1–7]	5 [2–7]*	5 [2–7]*			
SVRI (dynes·sec·cm <sup>-5</sup> /kg)	Normal	254.4 [170.4–471.5]	189.2 [95.5–500.2]	112.7 [72.0–199.4]	87.6 [67.3–133.4] <sup>A</sup>	<0.001	<0.001	0.183
	Acidosis	132.4 [112.1–239.3]*	110.2 [74.6–192.0]*	83.6 [58.3–123.0]	66.7 [41.3–99.1]* <sup>A</sup>			
PAP (mm Hg)	Normal	12 [9–13]	15 [11–17]	18 [17–21] <sup>AB</sup>	20 [16–23] <sup>AB</sup>	<0.001	<0.001	0.492
	Acidosis	19 [16–20]*	24 [21–25]* <sup>A</sup>	26 [22–27]* <sup>A</sup>	27 [23–29]* <sup>A</sup>			
PAOP (mm Hg)	Normal	5 [3–6]	5 [3–7]	5 [4–6]	4 [3–6]	<0.001	0.800	0.937
	Acidosis	11 [6–13]*	11 [7–13]*	10 [8–12]*	10 [8–12]*			
PVRI (dynes·sec·cm <sup>-5</sup> /kg)	Normal	26.7 [16.4–35.1]	26.0 [15.9–47.3]	23.3 [19.4–38.4]	24.4 [16.0–32.4]	0.661	0.811	0.201
	Acidosis	22.1 [13.5–33.6]	22.5 [13.8–34.9]	21.7 [14.7–34.1]	22.2 [13.8–33.0]			

CI, cardiac index; HR, heart rate; SVI, stroke volume index; SAP, systolic arterial pressure; MAP, mean arterial pressure, DAP, diastolic arterial pressure; RAP, right atrial pressure; SVRI, systemic vascular resistance index; PAP, pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; PVRI, pulmonary vascular resistance index. The rank transformation version of two-way ANOVA applies on condition and dobutamine treatment. Superscript A, B, and C show significant difference ( $P < 0.05$ ) from baseline, 5 µg/kg/min, and 10 µg/kg/min by Steel-Dwass, respectively. \* shows a significant difference ( $P < 0.05$ ) as compared with each dose in Normal condition.

## 289 **Discussion**

290

291 To the best of our knowledge, this study is the first to evaluate the dose-dependent  
292 cardiovascular function of colforsin in dogs. Colforsin had a cardiovascular action similar to  
293 that of dobutamine. It increased CI and HR and decreased SVRI in a dose-dependent manner.  
294 However, under acute respiratory acidosis, the rates of change in CI, HR, and SVRI were  
295 attenuated with both colforsin and dobutamine. Therefore, colforsin and dobutamine doses may  
296 have to be increased under respiratory acidosis.

297

### 298 **1) Cardiovascular effects of dobutamine and colforsin in the** 299 **Normal condition**

300 Dobutamine is a synthetic dopamine analog which stimulates beta-1, beta-2, and alpha-  
301 1 adrenoceptors in the cardiovascular system at doses approximating those used clinically (1–  
302 20 µg/kg/min) [2,9,10]. The inotropic activity of dobutamine is the result of stimulating both  
303 beta-1 and alpha-1 adrenoceptors in the myocardium. Furthermore, the beta-2 adrenoceptor-  
304 mediated vasodilatory effect of dobutamine is offset by alpha-1 adrenoceptor-mediated  
305 vasoconstrictor activity. Therefore, dobutamine increases CI and HR and decreases SVRI  
306 (inodilation) in a dose-dependent manner [11,12]. In the present study, dobutamine  
307 administration raised both CI and HR and lowered SVRI which corroborates previous reports.

308 Colforsin activates adenylate cyclase in cardiomyocytes and vascular smooth muscle  
309 without mediating catecholamine beta adrenoceptors. It increases cardiac contractility and  
310 reduces peripheral vascular resistance [13]. We used dobutamine as a positive control in the  
311 present study. At clinical doses, dobutamine induced dose-related inotropism and afterload  
312 reduction with a relative lack of chronotropism. These conditions are appropriate for the

313 management of patients with congestive heart failure. They could also improve renal blood  
314 flow by enhancing cardiac output and beta-2 adrenoceptor-stimulated vasodilation [12,14,15].  
315 The colforsin doses administered in the present study (0.3 µg/kg/min, 0.6 µg/kg/min, and 1.2  
316 µg/kg/min) were those required to increase the heart rate to a level equivalent to that induced  
317 by dobutamine in our preliminary study (data not shown). In the present study, colforsin  
318 administration increased CI and HR and decreased SVRI as did dobutamine. Therefore,  
319 colforsin could substitute for dobutamine as an inodilator and might be useful for the treatment  
320 of pathological conditions such as congestive heart failure.

321 Unlike dobutamine, colforsin did not increase the PAP. Vascular smooth muscle in the  
322 pulmonary artery was relaxed by beta adrenoceptor stimulation [16]. In terms of inodilator  
323 dose–response effects in rats, dobutamine increased systolic pulmonary artery pressure [17]. It  
324 also slightly elevated pulmonary vascular resistance in anesthetized dog [18]. Pulmonary  
325 hypertension is defined as pulmonary arterial systolic pressure > 30 mm Hg or pulmonary  
326 arterial mean pressure > 20 mm Hg [19]. In the present study, dobutamine increased the PAP  
327 in a dose-dependent manner. Dobutamine at 10 µg/kg/min elevated the mean PAP > 20 mm Hg  
328 in 1/6 dogs while 20 µg/kg/min dobutamine had the same effect on 3/6 dogs. In contrast,  
329 colforsin administration produced no pulmonary hypertension. Left-sided heart disease is the  
330 most common cause of pulmonary hypertension in humans and dogs [19,20]. In a study of 60  
331 dogs with pulmonary hypertension, 38 (63%) presented with degenerative mitral valve disease  
332 [21]. Other studies indicated that 14–31% of all dogs diagnosed with the latter disorder  
333 developed pulmonary hypertension [22,23]. Therefore, colforsin might be more efficacious  
334 than dobutamine in the treatment of severe mitral valve insufficiency accompanied by  
335 pulmonary hypertension. The effects of colforsin and dobutamine on the vascular smooth  
336 muscle of the pulmonary artery merit further investigation.

337

## 338 **2) Cardiovascular effects of dobutamine and colforsin in the** 339 **Acidosis condition**

340 In the present study, the acute respiratory acidosis canine model was induced by carbon  
341 dioxide inhalation [24]. The baseline pH at the time of dobutamine and colforsin administration  
342 was ~7.0. However, PaCO<sub>2</sub> slightly increased during the experiment (0.5 h for stabilization and  
343 3 h for measurement). Although the pH had slightly decreased by the end of the experiment,  
344 we believe that acute respiratory acidosis was induced in the dogs at ~pH 7.0.

345 Acute respiratory acidosis increases cardiac output and heart rate in dogs [25].  
346 Symptoms of early hypercapnia include nausea/vomiting, muscle twitching, extrasystoles, and  
347 sympathetic nervous system stimulation. In the present study, the plasma adrenaline and  
348 noradrenaline levels in the Acidosis condition were significantly higher than those in the  
349 Normal condition. Therefore, elevated catecholamines could increase cardiac output and stroke  
350 volume under acidosis. Hypercapnia also causes anesthesia and peripheral blood vessel dilation  
351 [25]. At baseline, hypercapnia might have decreased systemic vascular resistance under  
352 acidosis in the present study.

353 The affinity of catecholamine for the beta adrenoceptor decreases under acidosis [26].  
354 Therefore, the cardiovascular effects of catecholamines are attenuated. On the other hand,  
355 colforsin improved cardiac contractility in isolated and acid-perfused rat heart under acidosis  
356 [7]. It was also reported that the cAMP level in cardiac muscle cells was higher in response to  
357 colforsin than to catecholamines [7]. However, the cardiovascular effects of colforsin on CI,  
358 HR, and SVRI resembled those of dobutamine in the present study. The rates of change in these  
359 variables in response to both drugs were weaker under the Acidosis condition than under the  
360 Normal condition. As cardiomyocyte cAMP was not measured here, it could not be determined  
361 whether the same reaction was occurring as that reported in the previous study. Moreover,  
362 acidic perfusate rather than hypercapnia was considered in that study. Hypercapnia is anesthetic

363 and suppresses cardiovascular function [27,28]. Consequently, even if the same isoflurane dose  
364 was administered to both groups, anesthesia may have been more profound in the Acidosis  
365 group than in the Normal group because the former presented with hypercapnia. For this reason,  
366 the effects of colforsin and dobutamine may have been attenuated by hypercapnia.

367 Pulmonary arterial vasoconstriction occurs in response to alpha adrenoceptor the  
368 stimulation [29]. The alpha adrenoceptors in the pulmonary arteries have a high affinity for  
369 catecholamines such as noradrenaline. As the baseline noradrenaline concentration was high in  
370 the present study, the PAP was higher in the Acidosis condition than in the Normal condition.  
371 In the experimental induction of microembolic pulmonary hypertension, high dobutamine doses  
372 decreased pulmonary artery pressure [30]. In the present study, all dogs administered with  
373 dobutamine showed pulmonary hypertension > 20 mm Hg. Although certain dogs presented  
374 with pulmonary hypertension at high colforsin doses, their PAP was low relative to that induced  
375 by dobutamine. Even under acidosis, the influence of colforsin on pulmonary artery pressure  
376 was small compared with that of dobutamine. Therefore, the fact that colforsin had zero impact  
377 on pulmonary artery pressure may facilitate its application as an adjunct to (or replacement for)  
378 dobutamine.

379 Acute respiratory distress syndrome (ARDS) is life-threatening and caused by sepsis  
380 or a systemic inflammatory response. ARDS requires ventilator management in intensive care  
381 and lung protective ventilation is recommended [31]. A consequence of low tidal volume  
382 ventilation is an elevation in PaCO<sub>2</sub>. In humans, high PaCO<sub>2</sub> levels (> 70 mm Hg) may be  
383 tolerated (permissive hypercapnia). Nevertheless, heavier sedation or paralysis may be required  
384 to prevent patient-ventilator asynchrony [32,33]. Past evidence from experimental animal  
385 studies [24,34] and human clinical trials [31] suggest that lung-protective ventilation would  
386 also be warranted in veterinary patients. We set our acute respiratory acidosis model higher  
387 than that required for lung-protective ventilation in order to differentiate the drug effects clearly.



388 ARDS often causes pulmonary hypertension as a result of hypoxic pulmonary vasoconstriction  
389 and pulmonary blood vessel organization. In the present study, colforsin did not raise  
390 pulmonary artery pressure in the Acidosis condition. It was reported that colforsin attenuates  
391 bronchoconstriction- and pulmonary hypertension-induced serotonin infusion in dogs [35].  
392 Therefore, colforsin may be able to improve cardiac function in permissive hypercapnia with  
393 pulmonary hypertension more effectively than dobutamine. The application of colforsin for the  
394 treatment of pulmonary hypertension caused by mitral valve insufficiency and ARDS might be  
395 a new therapeutic strategy in both veterinary and human medicine.

396 In the present study, plasma glucose level was higher in the Acidosis condition than in  
397 the Normal condition at baseline. Catecholamines markedly increase plasma glucose levels  
398 [36,37]. Insulin secretion declines after alpha adrenoceptor activation but rises in response to  
399 beta-2 adrenoceptor activation [38]. The high baseline plasma glucose level in the Acidosis  
400 condition was positively correlated with high plasma adrenaline and noradrenaline levels.  
401 Although dobutamine stimulates beta-1, beta-2, and alpha-1 adrenoceptors [2,9], the  
402 dobutamine dosage administered in this present study did not affect plasma glucose level under  
403 the Normal condition. The effects of alpha-1 catecholamine may have been offset by the beta-  
404 2 catecholaminic action of dobutamine. The effects of colforsin on insulin secretion are  
405 unknown. Nevertheless, the colforsin dose administered in the present study had no effect on  
406 the plasma glucose level. Therefore, colforsin might be appropriate for diabetic patients whose  
407 cardiovascular function must be improved without raising their plasma glucose levels. In the  
408 future, the influence of colforsin administration on plasma insulin concentration should be  
409 investigated.

410 In the Acidosis condition, the baseline plasma potassium level was higher than that at  
411 the end of the experiment. Lactic acidosis is probably not associated with major intracellular  
412 shifts in potassium level. However, respiratory acidosis may influence the serum potassium

413 concentration [39]. In the Normal condition, neither dobutamine nor colforsin increased plasma  
414 potassium levels. Moreover, there was no significant difference in plasma potassium between  
415 the Normal and Acidosis conditions at baseline. Relative to the baseline, however, plasma  
416 potassium level was significantly higher in the Acidosis condition at the end of experiment.  
417 Plasma potassium level rose in 3.5 h (0.5 h for stabilization and 3 h for the experiment) after  
418 the induction of acute respiratory acidosis. One acidotic dog receiving dobutamine (potassium  
419 level = 7.5 mmol/L) showed atrial stasis at the end of the experiment. Since the sample size was  
420 small in this assay, we could not confirm the relationship between dobutamine and arrhythmia.  
421 On the other hand, no arrhythmia was observed in dogs receiving colforsin (maximum  
422 potassium level = 8.3 mmol/L). Although there was no atrial stasis, colforsin acted as an  
423 inodilator here. Colforsin also suppressed digitalis- and epinephrine-induced ventricular  
424 arrhythmia models in dogs [40]. Abnormal plasma potassium levels and arrhythmia are often  
425 observed in heart- and renal failure [41]. Neither colforsin nor dobutamine affected plasma  
426 potassium levels under eucapnia. Therefore, both drugs neither aggravate nor alleviate acidotic  
427 increases in plasma potassium. For these reasons, colforsin could substitute for dobutamine in  
428 heart- and renal failure therapy. In the future, the associations among colforsin, plasma  
429 potassium level, and arrhythmia in these diseases should be investigated.

430         Some dogs presented with transient nausea or vomiting during recovery from both  
431 drugs. Dobutamine has provoked nausea, headache, vomiting, and dyspnea [2]. To the best of  
432 our knowledge, adverse effects have not been reported for colforsin. Nevertheless, it still may  
433 have side effects similar to those of dobutamine. The aim of the present study was to investigate  
434 the cardiovascular effects of colforsin under acidosis. Since the dose administered was  
435 impractical, many side effects may have been induced. In future research, we could endeavor  
436 to optimize the dosage of colforsin which would improve cardiovascular function in the  
437 presence of respiratory- or other acidosis. Certain dogs under the Acidosis condition showed

438 miosis at the end of the experiment. Miosis occurs when intracranial pressure increases. Since  
439 hypercapnia increases cerebral blood flow [42], it may have also elevated intracranial pressure  
440 and induced miosis. Dogs presenting with miosis returned to normal pupillary diameter within  
441 6 h after the experiment. No other neurological complications were observed. Although acute  
442 respiratory acidosis was maintained for 3.5 h in the present study, pupil size should be verified  
443 in respiratory acidosis and permissive hypercapnia in a clinical setting.

444 We conducted this study assuming that pulmonary edema or ARDS may complicate  
445 respiratory acidosis. However, there were certain limitations here. Although oxygenation is  
446 impaired in pulmonary edema and ARDS, we did not conduct this experiment under hypoxemia  
447 which stimulates the sympathetic nervous system and enhances cardiovascular function. We  
448 wanted to clarify the cardiovascular effects of colforsin in normal dogs. Therefore, we  
449 conducted this experiment with 100% oxygen carrier gas. Next, we used healthy dogs free of  
450 heart or lung disease. Dogs with severe mitral valve insufficiency causing pulmonary edema or  
451 ARDS already have depressed cardiorespiratory function. Therefore, administering colforsin  
452 and dobutamine to these patients may produce different cardiovascular effects. Further studies  
453 are needed to establish the effects of colforsin and dobutamine on these disease models and  
454 clinical cases and to verify the safety and efficacy of colforsin. In turn, these findings could be  
455 adapted to human medicine.

456

## 457 **Conclusions**

458 The cardiovascular effects of colforsin and dobutamine are similar in healthy beagles  
459 under isoflurane anesthesia. In acute respiratory acidosis induced by carbon dioxide inhalation,  
460 cardiovascular function was enhanced by endogenous catecholamine secretion. In addition, the  
461 rates of change in CI, HR, and SVRI caused by colforsin and dobutamine administration were  
462 attenuated. Therefore, it may be necessary to increase the colforsin and dobutamine doses under

463 respiratory acidosis relative to those administered under the normal condition. Since colforsin  
464 had little effect on the PAP, it may be more suitable as an inodilator than dobutamine in the  
465 treatment of diseases which increase the PAP. Our next steps are to induce a pulmonary  
466 hypertension canine model, confirm the effects of colforsin on it, adapt colforsin administration  
467 for patients with pulmonary hypertension in our institution, and compare its efficacy with that  
468 of existing catecholamines.

469

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