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1 Scaling of cardiac morphology is interrupted by birth in the developing sheep *Ovis aries*

2

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19 Running head: Scaling of cardiac morphology in sheep

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27 Abstract

28 Scaling of the heart across development can reveal the degree to which variation in cardiac morphology
29 depends on body mass. In this study, we assessed the scaling of heart mass, left and right ventricular
30 masses, and ventricular mass ratio, as a function of eviscerated body mass across fetal and postnatal
31 development in Horro sheep *Ovis aries* (~50-fold body mass range; $N = 21$). Whole hearts were extracted
32 from carcasses, cleaned, dissected into chambers, and weighed. We found a biphasic relationship when
33 heart mass was scaled against body mass, with a conspicuous ‘breakpoint’ around the time of birth,
34 manifest not by a change in the scaling exponent (slope), but rather a jump in the elevation. Fetal heart
35 mass (g) increased with eviscerated body mass (M_b , kg) according to the power equation $4.90M_b^{0.88 \pm 0.26}$ (\pm
36 95% CI), whereas postnatal heart mass increased according to $10.0M_b^{0.88 \pm 0.10}$. While the fetal and postnatal
37 scaling exponents are identical (0.88) and reveal a clear dependence of heart mass on body mass, only the
38 postnatal exponent is significantly less than 1.0, indicating the postnatal heart becomes a smaller
39 component of body mass as the body grows, which is a pattern found frequently with postnatal cardiac
40 development among mammals. The rapid doubling in heart mass around the time of birth is independent
41 of any increase in body mass and consistent with the normalization of wall stress in response to abrupt
42 changes in volume loading and pressure loading at parturition. We discuss variation in scaling patterns of
43 heart mass across development among mammals, and suggest that the variation results from a complex
44 interplay between hard-wired genetics and epigenetic influences.

45

46 Key words: allometry, biphasic, cardiac, fetal, morphogenesis, ontogeny

47 Introduction

48 Cardiogenesis, growth and remodeling of the heart are driven by an orchestrated program of gene
49 activation and repression under the precise spatial and temporal control of transcription factors and
50 microRNAs (Roche et al., 2013; Sylva et al., 2014). Biomechanical forces exerted by blood on the walls
51 of the heart shape the phenotype by inducing gene expression and differentiation necessary for normal
52 developmental patterning (Lindsey et al., 2014). The embryonic heart forms when cardiac progenitor
53 cells arise from the mesoderm and establish in the cranial region of the embryo, where they arrange to
54 form the cardiac crescent, before coalescing and fusing into a single cardiac tube that undergoes looping
55 and ‘ballooning’ of the chambers (Christoffels et al., 2000; Moorman & Christoffels, 2003). The fetal
56 heart takes form when the chambers and outflow tract undergo septation, a compact myocardium is laid
57 down, and leaflet valves develop (Fig 1a). The fetal heart nonetheless maintains a small fissure through
58 the atrial septum, the *foramen ovale*, which allows for right-to-left atrial shunting, while the thicker
59 ventricular septum completely separates the left and right ventricles (Sylva et al., 2014). A compact
60 myocardium forms at the epicardial side of the developing fetal heart (Ieda et al., 2009), and soon after
61 the newly formed coronary vascular network connects with the base of the aorta, becoming functional,
62 and allows perfusion of the compact cardiac tissue (Tomanek, 1996; Farrell, 1997; Farrell et al., 2012).
63 Facilitated by an increasing role of the sinoatrial and atrioventricular nodes in the fetal heart, a mature
64 apex-to-base activation sequence of the septated ventricles arises (Sedmera & Ošřádal, 2012). Leaflet
65 valves form at the atrioventricular canals, and at the aortic and pulmonary outflow tracts, ensuring the
66 unidirectional flow of fetal blood (Sedmera & Ošřádal, 2012). Across gestation, the fetal heart increases
67 in absolute mass, and increases its capacity to generate both absolute blood flow (Rudolph & Heymann,
68 1970) and blood pressure (Dawes et al., 1980; Kitanaka et al., 1989; Giussani et al., 2005).

69 At birth, the transition from placental to pulmonary gas exchange changes significantly the flow
70 and pressure requirements of the heart. The placental circulation is eliminated, and there is closure of the
71 *foramen ovale* between the atrial chambers, closure of the *ductus arteriosus* channel between pulmonary
72 artery and aorta, and closure of the *ductus venosus* channel that bypasses the liver (Rudolph, 1970;

73 Thornburg et al., 1997). With this re-plumbing, the left and right ventricular chambers adjust from
74 working against the same blood pressure with different blood flow outputs *in utero* to working against
75 significantly different blood pressures but with identical blood flow outputs *post utero*. The postnatal
76 heart also has to deal for the first time with the effects of gravity. Measurements from sheep, taken before
77 and soon after birth, document an increase in the mass-specific cardiac outputs of both ventricular
78 chambers, with the left ventricle in particular increasing from ca. 150 to 300 – 450 mL min⁻¹ kg⁻¹ over this
79 brief perinatal period (original data or summarised in Klopfenstein & Rudolph, 1978; Lister et al., 1979;
80 Anderson et al., 1981; Heymann et al., 1981; Rudolph, 1985; Morton et al., 1987; Stopfkuchen, 1987;
81 Grant, 1999). The increase in left ventricular cardiac output is achieved in large part by an increase in the
82 mass-specific stroke volume, which doubles, from ca. 1 to 2 mL kg⁻¹. The large increase in left
83 ventricular mass-specific stroke volume at birth is congruent with reports that heart growth after birth is
84 disproportionately in favour of the left ventricle over the right ventricle (Lee et al., 1975), a process
85 thought to involve accelerated proliferation and enlargement of the left ventricular cardiomyocytes
86 (Smolich et al., 1989). As the postnatal heart continues to grow and mature to adulthood, there is an
87 associated rise in absolute cardiac output (Woods Jr et al., 1978), although the increase in systemic
88 arterial blood pressure is comparatively minor (Berman & Christensen, 1983).

89 Despite recent advancements to our qualitative understanding of cardiac development (Roche et al.,
90 2013; Sylva et al., 2014), our quantitative understanding has not kept pace, in part because of our failure
91 to account for the non-linear effects of body size on cardiac structure and function (Calder III, 1996;
92 Batterham et al., 1999; Chantler et al., 2005). One way of describing cardiac structure and function is by
93 scaling analysis (allometry), which relates an anatomical or physiological cardiac variable (Y) to body
94 mass (M_b), usually by a power equation, $Y = aM_b^b$, where a (the coefficient) represents the elevation of
95 the curvilinear line (value of Y when $M_b = 1$), and b (the exponent) describes the shape of the curvilinear
96 line (Fig 1b). If $b = 1$, then Y increases in direct proportion to M_b ; if $b = 0$, Y is independent of M_b ; if $1 >$
97 $b > 0$, Y increases with a curve that has a decreasing slope against M_b ; if $b > 1$, Y increases with a curve
98 with an increasing slope against M_b ; and if $0 > b > -1$, Y decreases with a curve that has a decreasing

99 slope against M_b . Logarithmic transformation of the data straightens the line for statistical interrogation,
100 and the rearranged equation becomes, $\log Y = \log a + b \log M_b$, where $\log a$ now represents the elevation of
101 the linearized relationship (y-intercept, value of $\log Y$ when $\log M_b = 0$), and b is unchanged but now
102 defines the slope of the linearized relationship. Thus, in reference to b , the terms ‘slope’ and ‘exponent’
103 are often used interchangeably.

104 Previous scaling analyses on a variety of mammal species reveal that heart mass increases with
105 body mass across postnatal development according to a power equation with a scaling exponent (slope)
106 that usually is less than 1.0, meaning that the ratio of heart mass to body mass decreases as the body
107 grows across postnatal life (summarised in Snelling et al., 2015a). This supports earlier observations that
108 the neonate heart is relatively larger than the adult heart in several species of laboratory and domestic
109 mammals (Lee et al., 1975). Scaling studies that have broadened the analysis to include the fetal life
110 stage of placental mammals and the in-pouch life stage of marsupial mammals have led to the suggestion
111 that heart mass has a biphasic relationship with body mass, with a ‘breakpoint’ at which the exponent
112 (slope) changes at birth and at pouch exit in these respective groups. In humans, the heart mass exponent
113 is reported to be 1.19 across fetal development and 0.89 across postnatal development (Hirokawa, 1972),
114 which implies that before birth, but not after birth, cardiac growth outpaces that of body mass. However,
115 that study obtained its data from spontaneously aborted fetuses, many with known cardiopulmonary
116 disease. In giraffes *Giraffa camelopardalis*, the fetal heart mass exponent is 1.03 and the postnatal
117 exponent is 0.90 (Mitchell & Skinner, 2009). However, adult giraffes are unusual in that their long
118 vertical heart-to-head distance requires them to generate exceptionally high mean central arterial blood
119 pressures, achieved by a relatively thick-walled left ventricle (Smerup et al., 2016), but it is unclear when
120 those high blood pressures first appear during development. Two studies give different patterns of
121 cardiac growth in two different species of marsupial, which give birth to extremely altricial, ectothermic-
122 young that develop within a pouch. For the western grey kangaroo *Macropus fuliginosus*, the in-pouch
123 heart mass exponent is 1.10 and the post-pouch exponent is 0.77 (Snelling et al., 2015a). However, in the
124 tammar wallaby *Macropus eugenii*, heart mass scales in perfect isometry with body mass (exponent of

125 1.0) across the full scope of development with no detectable breakpoint between in-pouch and post-pouch
126 life stages (Hulbert et al., 1991). Thus, there is reason to question the generality of a biphasic scaling
127 pattern of heart mass growth across development in mammals.

128 In this study, we assessed the scaling of cardiac morphology across fetal and postnatal development
129 in Horro sheep *Ovis aries*, a common breed of Ethiopia readily available to us. Using this sheep as a
130 model, we seek further evidence for or against a biphasic scaling pattern of heart mass across
131 development, and we investigate if the apparent doubling of left ventricular mass-specific stroke volume
132 at birth is reflected in the gross morphology of the heart. Scaling relationships are presented for whole
133 heart mass, left ventricular (LV) mass, right ventricular (RV) mass, and ventricular mass ratio (RV/LV).

134 Materials and methods

135 *Animal carcasses*

136 This study was approved by the Animal Ethics Committees of the University of the Witwatersrand
137 (2017/05/33/0) and Addis Ababa University (427/09/2017). In cooperation with local abattoirs in Addis
138 Ababa, we purchased whole hearts extracted from carcasses of Horro sheep *Ovis aries*, a short-fleeced,
139 medium-sized breed from Ethiopia, with nothing unusual in its morphology (Gizaw et al., 2008). The
140 Horro sheep were supplied by nearby farms, from a mid-altitude region, approximately 2500 m above sea
141 level. Typically, the Horro sheep has a birth body mass of 2.7 kg, a weaning body mass of 12 kg (ca. 93
142 days), a mature body mass of 25 – 35 kg, and an average litter size of 1.34 (Abegaz et al., 2000; Ermias et
143 al., 2006). The abattoir workers killed each postnatal sheep according to their standard practice, before
144 eviscerating the carcass, removing the gastrointestinal tract and its contents, but leaving behind all other
145 internal organs. Eviscerated body mass was recorded to the nearest 0.01 kg on a calibrated digital strain
146 gauge scale (PK-110; AWS, Cumming, GA, USA). The abattoir workers then removed the hearts, and
147 we sealed them in plastic zip-lock bags, transported them back to the laboratory, and froze them until day
148 of dissection. In addition, the abattoirs supplied us with fetal carcasses. For consistency, we sealed and
149 transported these fetal carcasses in plastic zip-lock bags and froze them until day of dissection, at which
150 time we weighed them, removed the gastrointestinal tract, and reweighed them, and then removed the
151 heart for dissection. We recorded their intact total body mass and their eviscerated body mass to the same
152 precision using the same strain gauge scale as for the postnatal sheep.

153

154 *Heart dissections*

155 We obtained 30 hearts from sheep carcasses, from which we measured 10 fetal hearts and 11 postnatal
156 hearts. We excluded 9 hearts that were either not fully formed (e.g., incomplete septation) or not
157 extracted whole by the abattoir workers (e.g., missing atrial chamber). We emptied the chambers of
158 congealed blood, removed major blood vessels, trimmed any excess fat, and dissected the chambers from
159 one another. For both fetal and postnatal hearts, we excised the LV free wall plus the interventricular

160 septum ('left ventricle'), and the RV free wall ('right ventricle'), following what have become standard
161 procedures (Fulton et al., 1952; Keen, 1955; Joyce et al., 2004; Snelling et al., 2016). The myocardial
162 mass of each chamber was determined by weighing to the nearest 0.01 g on a calibrated analytical balance
163 (ADP-2100; Adam Equipment, Milton Keynes, UK) and the chamber masses summed to provide whole
164 heart mass.

165

166 *Statistical analyses*

167 Scaling relationships are presented to describe the change in whole heart mass (atria + ventricles), LV
168 mass, RV mass, and ventricular mass ratio (RV/LV), each as a function of eviscerated body mass, across
169 fetal and postnatal development in Horro sheep. The scaling relationships are in the form of a power
170 equation, $Y = aM_b^{b \pm 95\% \text{ CI}}$, where Y is the cardiac variable of interest, a is the scaling coefficient
171 (elevation), b is the scaling exponent (slope of the log-transformed relationship), M_b is the eviscerated
172 body mass in kg, and CI stands for confidence interval. To analyse the scaling relationships statistically,
173 we took the \log_{10} of the cardiac variable and the \log_{10} of eviscerated body mass, and applied ordinary
174 least-squares linear regressions to the log-transformed data (Smith, 2009; Kilmer & Rodriguez, 2017). To
175 determine whether heart mass had a biphasic relationship with body mass, we performed a broken stick
176 analysis by fitting a series of two-phase linear regressions to the log-transformed data. We identified the
177 breakpoint as the intersection that minimised the sum for both 'sticks' of the regressions' residual sums of
178 squares (Yeager & Ultsch, 1989; Mueller & Seymour, 2011). The slopes and elevations of the
179 regressions then were compared between fetal and postnatal life stages by ANCOVA (Zar, 1998) by
180 means of dedicated statistical software (Prism 7; GraphPad Software, La Jolla, CA, USA).

181 Results

182 *Scaling of whole heart mass*

183 When the fetal and postnatal life stages of Horro sheep are combined, whole heart mass increases with
184 eviscerated body mass according to a power equation with a scaling exponent of 1.12 ± 0.11 ($\pm 95\%$ CI)
185 (Fig 2a). However, this analysis obscures a clear biphasic relationship, driven by a doubling in whole
186 heart mass around the time of birth (Fig 2b). The increase appears to be real, not a statistical artefact, and
187 is confirmed by the broken stick analysis, which identified the breakpoint at birth. When considered
188 separately, the fetal and postnatal life stages have the same scaling exponent for heart mass against
189 eviscerated body mass (0.88 ± 0.26 and 0.88 ± 0.10 , respectively; ANCOVA, $P = 0.96$), but the scaling
190 elevations are markedly and statistically different ($P < 0.0001$; Table 1). While the identical exponent
191 (0.88) seems to imply that neither fetal nor postnatal cardiac growth keeps pace with body mass, the fetal
192 heart mass exponent is not significantly different from isometry (95% CI overlaps 1.0), and only the
193 postnatal exponent shows statistical hypoallometry (95% CI less than 1.0).

194

195 *Scaling of LV and RV masses*

196 The scaling exponents for LV mass against eviscerated body mass are statistically indistinguishable
197 across fetal and postnatal development, 0.90 ± 0.35 and 0.90 ± 0.11 , respectively (ANCOVA, $P = 0.99$),
198 but the scaling elevations are significantly different ($P < 0.0001$; Fig 3a). Likewise, the exponents for RV
199 mass against eviscerated body mass are statistically indistinguishable across fetal and postnatal
200 development, 0.93 ± 0.23 and 0.92 ± 0.13 , respectively ($P = 0.87$), but in this case of the RV, the
201 elevations are not statistically different ($P = 0.07$; Fig 3b). Therefore, the 2.0-fold increase in whole heart
202 mass around the time of birth is due to an increase in LV mass (2.4-fold) rather than RV mass (1.3-fold).
203 Indeed, the ventricular mass ratio (RV/LV) changes around the time of birth (Fig 3c), from an average
204 ratio of 0.60 ± 0.10 across fetal development (exponent of 0.04 ± 0.30) to an average ratio of 0.33 ± 0.03
205 across postnatal development (exponent of 0.02 ± 0.15).

206 Discussion

207 This study used scaling analysis to track change in cardiac morphology, as a function of eviscerated body
208 mass, across fetal and postnatal development in Horro sheep. Previous scaling studies of humans
209 (Hirokawa, 1972), giraffes (Mitchell & Skinner, 2009), and kangaroos (Snelling et al., 2015a) revealed a
210 biphasic relationship between heart mass and body mass, with heart mass increasing with a relatively
211 steep exponent (slope) across fetal or in-pouch life, before transitioning to a shallower exponent across
212 postnatal or post-pouch life. However, that pattern was not apparent in Horro sheep. Instead, the scaling
213 exponents for heart mass are identical before and after parturition, and the breakpoint around the time of
214 birth is manifest as a jump in the elevation. The resetting of elevation is almost entirely due to a rapid
215 increase in LV mass during the perinatal period. We begin this discussion by assessing the likely effect
216 of using eviscerated body mass, rather than intact total body mass, as the independent variable in the
217 scaling analysis. Next, we show how the rapid increase in heart mass around the time of birth is
218 consistent with well-documented changes in mass-specific stroke volumes and arterial blood pressures
219 recorded from near-term fetal sheep and 1-week-old neonatal lambs. Lastly, we discuss the implications
220 of heart mass scaling across fetal and postnatal development and its relationship to whole body
221 physiology.

222

223 *Effect of using eviscerated body mass in the scaling analysis*

224 Our postnatal Horro sheep were sourced as fresh, eviscerated carcasses from abattoirs and, as such, our
225 eviscerated body mass omits the masses of the gastrointestinal tract, its foodstuff contents, and some
226 blood. A previous study on the postnatal Horro sheep indicates that eviscerated body mass is
227 approximately 25% lower than intact total body mass (Ermiyas et al., 2006), although it is unclear if this
228 proportion changes with postnatal body growth. Our fetal sheep were weighed before and after
229 evisceration, with eviscerated body mass 8% lower than intact total body mass, and the deficit
230 independent of fetal body growth. If, instead, we use fetal total body masses and if we apply a +25%
231 adjustment to our postnatal eviscerated body masses, the exponent describing heart mass as a function of

232 body mass across fetal and postnatal life stages combined decreases from 1.12 ± 0.11 to 1.05 ± 0.08 (Fig
233 2a). However, the adjustment does not affect the scaling exponents of the fetal and postnatal life stages
234 when they are treated separately, but the magnitude of the jump in heart mass around the time of birth is
235 reduced from 2.0-fold to 1.7-fold (Fig 2b). Thus, our observation concerning the different fetal and
236 postnatal scaling elevations is not an artefact resulting from the use of eviscerated body mass rather than
237 total body mass.

238

239 *Scaling of cardiac morphology is interrupted by birth*

240 At birth, the precocial neonate for the first time must satisfy the energy-intensive tasks of endothermy,
241 independent locomotion, and independent nutrition. The heart and circulation remodel from parallel
242 circuits incorporating the placenta to an in-series circuit via the lungs. Associated with this remodeling,
243 the left and right ventricular chambers transition from working against the same blood pressure with
244 different blood flow outputs *in utero* to working against significantly different blood pressures but with
245 identical blood flow outputs *post utero* (Rudolph, 1970; Thornburg et al., 1997). It is congruent then that
246 our scaling analysis reveals a biphasic relationship between heart mass and body mass in Horro sheep,
247 with a breakpoint occurring around the time of birth, in the form of a 2.0-fold increase in heart mass,
248 driven primarily by a 2.4-fold increase in LV mass (Fig 3a), and supplemented by a not-statistically-
249 significant 1.3-fold increase in RV mass (Fig 3b). Evidence for the capacity of the perinatal heart to
250 rapidly gain myocardial mass, independent of any increase body mass, comes primarily from studies of
251 near-term fetal sheep, where experimentally-increased RV wall stress levels, induced by partial occlusion
252 of the pulmonary artery, elicit a 1.3 to 1.7-fold increase in mass-specific heart mass within 7 – 10 days
253 (Barbera et al., 2000; Segar et al., 2013). The rapid increase in LV mass around the time of birth in our
254 Horro sheep also aligns with two previous reports of a sharp ~1.3-fold increase in LV end-diastolic and
255 end-systolic linear dimensions in near-term fetal sheep compared to 2-day-old neonatal lambs
256 (Kirkpatrick et al., 1973; Anderson et al., 1984). Although the two studies did not consider the effects of
257 body size, a 1.3-fold increase in linear dimensions of the heart, raised to the third power, equates to a 2.2-

258 fold increase in volumetric dimensions of the heart, which we assume greatly exceeds the increase in
259 body size over the first two days of postnatal life. It has been suggested that at birth, the removal of
260 constraints that are caused by tissues surrounding the heart (e.g., fluid-filled lungs) allows for a near-
261 immediate increase in LV end-diastolic dimensions and preload, and thus facilitates increased LV stroke
262 volume in the neonate (Grant, 1999; Grant et al., 2001).

263 The biphasic scaling pattern of heart mass across development in Horro sheep, characterized by a
264 rapid increase in heart mass around the time of birth, and independent of any increase in body mass, is not
265 without precedent. Indeed, a recent scaling study showed a conspicuous breakpoint effected by a rapid
266 1.6-fold increase in the elevation of heart mass at hatching, in another precocial endotherm with a four-
267 chambered heart, the Pekin duck *Anas platyrhynchos domestica* (Fig 4a; Sirsat et al., 2016). Nonetheless,
268 the breakpoint in the scaling elevation of Horro sheep differs from what we found when we reanalyzed
269 published heart mass and body mass data for sheep of mixed Western breeds. That analysis revealed a
270 breakpoint in the scaling exponent rather than elevation around the time of birth, with a relatively steep
271 gain in heart mass across fetal development transitioning to a hypoallometric trajectory across postnatal
272 development (Fig 4b; Jonker et al., 2015). Likewise, two previous studies on placental mammals
273 (humans and giraffes) reported a breakpoint in the scaling exponent around the time of birth (Hirokawa,
274 1972; Mitchell & Skinner, 2009). These differences among studies warrant future work because the
275 substantial widening of the ventricular mass ratio (RV/LV) around the perinatal period [from 0.60 in the
276 fetal heart to 0.33 in the postnatal heart, as a result of the disproportionate increase in LV mass compared
277 to RV mass] has been reported widely for other placental mammals (Lee et al., 1975). In humans, the
278 ratio decreases from 0.8 at birth, to 0.6 within a few days after birth, and to 0.4 by 3 months of age (Keen,
279 1955; Joyce et al., 2004).

280 The rapid increase in LV mass, but not RV mass, that we observed in Horro sheep is consistent
281 with the different changes in volume loading and pressure loading on the chambers at birth. In
282 accordance with the Principle of Laplace, an approximate model for mean circumferential wall stress (σ)
283 of a thick-walled sphere is given by the relationship, $\sigma = r_i P_i / 2h$, where r_i is internal radius, P_i is

284 transmural pressure and h is wall thickness (Mirsky, 1974; Westerhof et al., 2010). Although the
285 chambers of the heart are not exactly thick-walled spheres, and although their geometry likely changes
286 with remodeling at birth, this formula nonetheless shows that, if other variables are held constant, an
287 increase in either volume loading ($\uparrow r_i$) or pressure loading ($\uparrow P_i$) on the chamber walls, would require an
288 increase in wall thickness ($\uparrow h$) to spread the additional load and normalize mean circumferential wall
289 stress (Seymour & Blaylock, 2000). Therefore, we posit that the 2.4-fold increase in LV mass at around
290 the time of birth, which occurs independent of changes in body mass, likely reflects an increase in LV
291 wall thickness to normalize wall stress in response to an abrupt increase in volume loading (i.e., end-
292 diastolic volume). The evidence for the increase in volume loading comes from studies that report an
293 increase in mass-specific LV stroke volume around the time of birth (assuming constant ejection
294 fraction), which doubles from approximately 1 mL kg⁻¹ in near-term fetal sheep to 2 mL kg⁻¹ in 1-week-
295 old neonatal lambs (original data or summarised in Klopfenstein & Rudolph, 1978; Lister et al., 1979;
296 Anderson et al., 1981; Morton et al., 1987; Stopfkuchen, 1987; Grant, 1999). Indeed, a comparable
297 increase in LV mass-specific stroke volume has been recorded from near-term fetal sheep subject to
298 artificial positive pressure ventilation simulating birth (Teitel et al., 1987). To a slightly lesser extent, the
299 increase in LV mass around the time of birth also is likely a consequence of an increase in LV wall
300 thickness due to an increase in pressure loading. The evidence for the increase in pressure loading comes
301 from studies that report an increase in left-sided blood pressure from approximately 50 mmHg in near-
302 term fetal sheep to 70 mmHg in newborn lambs (summarised in Grant, 1999; Jonker & Louey, 2016).
303 Once again in accordance with the Principle of Laplace, we posit that the lack of a significant increase in
304 RV mass around the time of birth likely results from the absence of a substantial change in RV wall
305 thickness, at least partly due to the counteracting effects of an increase in volume loading and a decrease
306 in pressure loading. On the one hand, RV volume loading likely increases around the time of birth based
307 on studies that report a ~1.2-fold increase in mass-specific RV stroke volume (assuming constant ejection
308 fraction), from approximately 1.7 mL kg⁻¹ in near-term fetal sheep to 2 mL kg⁻¹ in 1-week-old neonatal
309 lambs (original data or summarised in Klopfenstein & Rudolph, 1978; Lister et al., 1979; Anderson et al.,

310 1981; Morton et al., 1987). On the other hand, and probably of greater importance, RV pressure loading
311 likely decreases around the time of birth due to an abrupt decrease in right-sided blood pressure, which
312 drops from approximately 50 mmHg in near-term fetal sheep to 20 – 30 mmHg in newborn lambs
313 (summarised in Grant, 1999; Jonker & Louey, 2016). While we have left aside the important influence of
314 the geometry of the ventricular chambers, it is worth noting that the postnatal RV remains relatively thick
315 walled, given the low pressure it generates compared to the postnatal LV, probably as a consequence of
316 its larger radius of curvature, which places the RV at a disadvantage in the development of pressure
317 (Huisman et al., 1980).

318

319 *Scaling of cardiac morphology across fetal and postnatal life*

320 The properties of the scaling of heart mass across fetal and postnatal development have other
321 physiological implications. In Horro sheep, fetal heart mass scales against body mass with a somewhat
322 shallow exponent of 0.88, not quite statistically different from 1.0 in our modest sample. However,
323 across fetal development, the maturation of cardiomyocyte ultrastructure will have implications for
324 cardiac function. Studies on the hearts of sheep and other placental mammals collectively show that
325 across fetal development (and sometimes extending into the neonatal period), there is a general
326 improvement in the alignment and organization of the myofilaments and myofibrils, and often a notable
327 increase in the volume densities of the myofibrils and mitochondria (Canale et al., 1986). Those changes
328 are concomitant with an increased contractile performance of isolated strips of myocardium and likely
329 contribute to the augmented functional performance of the whole heart (Canale et al., 1986; Smolich,
330 1995; Anderson, 1996). Indeed, a previous scaling analysis has shown maturation of cardiomyocyte
331 ultrastructure occurring during in-pouch development of the marsupial western grey kangaroo (Snelling et
332 al., 2015b). Myofibril and mitochondrial volume densities increase with respective scaling exponents of
333 0.13 ± 0.06 and 0.04 ± 0.03 , likely facilitating an increase in the functional performance of the heart as
334 the growing in-pouch young develop endothermy and become independently mobile. If a similar
335 maturation of cardiomyocyte ultrastructure and function occurs during fetal development of the Horro

336 sheep, then it will provide some compensation for the shallow scaling of fetal heart mass. To date, no
337 study has examined the scaling of cardiac ultrastructure, nor its relationship to cardiac performance and
338 whole body metabolic requirements, across the fetal and postnatal development of any placental mammal.

339 The hypoallometry of heart mass against body mass across the postnatal and post-pouch
340 development of mammals is common, although the range of scaling exponents is surprisingly broad,
341 typically from 0.7 to 1.0 (summarised in Snelling et al., 2015a). The present study with Horro sheep
342 revealed a postnatal exponent of 0.88, a value that is significantly lower than 1.0. An even shallower
343 exponent of 0.78 from sheep of mixed Western breeds implies that a more severe hypoallometry of
344 postnatal heart mass is possible (Jonker et al., 2015). In the marsupial western grey kangaroo, heart mass
345 scales with a hypoallometric exponent of 0.77 across post-pouch development (Snelling et al., 2015a)
346 despite the cardiomyocytes attaining ultrastructural maturity at pouch exit (Snelling et al., 2015b). The
347 reason why the growing heart tends to become relatively smaller as body mass increases across postnatal
348 development is probably varied and complex. Nevertheless, these comparisons seem to reinforce our
349 overall finding that there is much variation in the scaling patterns of heart mass against body mass across
350 development among mammals. The diversity of the observed scaling patterns likely arises from the
351 complex interplay between hard-wired genetic growth and maturation of the heart, and the influence of
352 epigenetic factors on the phenotype of the heart. Because of this interplay, we cannot yet generalize on
353 the exact scaling of either form or function of the heart across development, or the rationale behind the
354 scaling.

355

356 *Conclusions*

357 In this study, we use Horro sheep to demonstrate the utility of scaling in teasing apart changes in cardiac
358 morphology that are related to changes in body mass, and those that occur independently of body mass, at
359 different stages of development. We found that heart mass scales against eviscerated body mass with an
360 identical exponent of 0.88 over both the fetal and the postnatal life stages, but that the scaling elevations
361 differ significantly due to a rapid doubling in heart mass around the time of birth. This increase in heart

362 mass occurs independent of any change in body mass, and appears congruent with the normalization of
363 wall stress in response to changing volume loading and pressure loading on the ventricular walls at
364 parturition. We also show that the pattern of scaling of heart mass against body mass across development
365 among mammals varies greatly, likely resulting from a complex interplay between hard-wired genetics
366 and epigenetic influences.

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374

375 Author contributions

376 All authors contributed significantly to the study.

377

378 Conflict of interest

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

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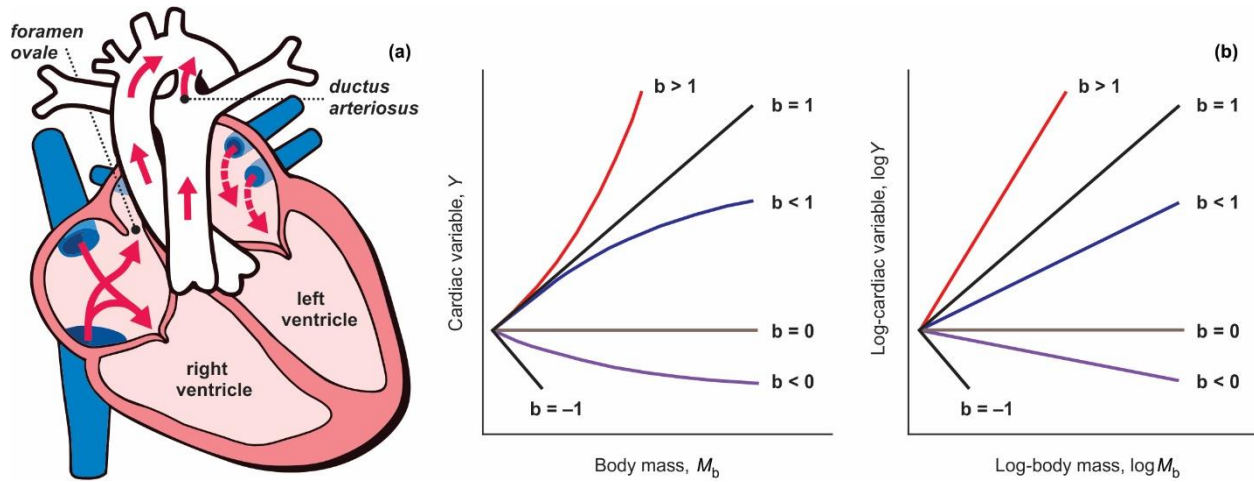
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511 Tables

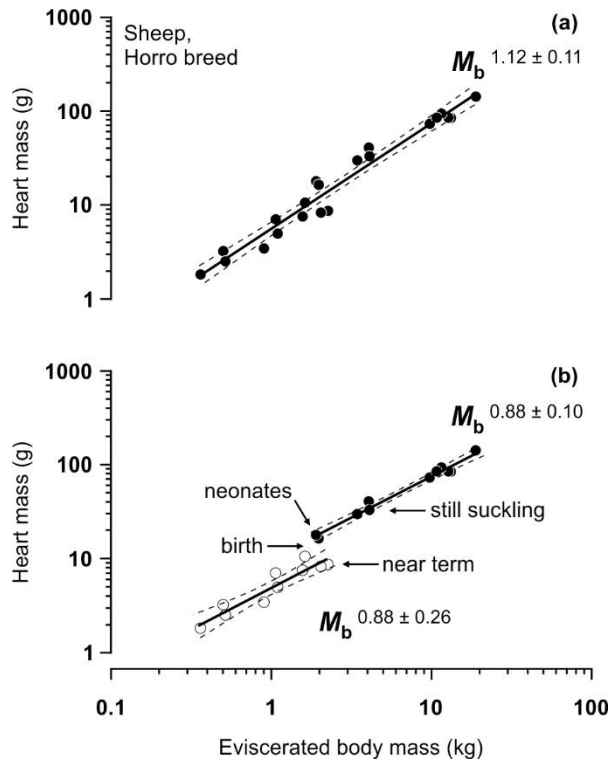
512 **Table 1.** Scaling relationships for whole heart mass (atria + ventricles), LV mass, RV mass, and ventricular mass
 513 ratio (RV/LV), each as a function of eviscerated body mass (gastrointestinal tract removed), in fetal ($N = 10$) and
 514 postnatal ($N = 11$) Horro sheep *Ovis aries* analysed in this study.

| | Fetal | Postnatal | ANCOVA comparisons of slope and (elevation) |
|----------------------|---|---|--|
| Whole heart mass (g) | $4.90M_b^{0.88 \pm 0.26}$ $r^2 = 0.88, P < 0.0001$ | $10.0M_b^{0.88 \pm 0.10}$ $r^2 = 0.98, P < 0.0001$ | $F_{1,17} = 2.6 \times 10^{-3}, P = 0.96$ ($F_{1,18} = 34.6, P < 0.0001$) |
| LV mass (g) | $2.69M_b^{0.90 \pm 0.35}$ $r^2 = 0.81, P < 0.001$ | $6.34M_b^{0.90 \pm 0.11}$ $r^2 = 0.97, P < 0.0001$ | $F_{1,17} = 8.3 \times 10^{-7}, P = 0.99$ ($F_{1,18} = 31.2, P < 0.0001$) |
| RV mass (g) | $1.57M_b^{0.93 \pm 0.23}$ $r^2 = 0.92, P < 0.0001$ | $2.01M_b^{0.92 \pm 0.13}$ $r^2 = 0.96, P < 0.0001$ | $F_{1,17} = 0.028, P = 0.87$ ($F_{1,18} = 3.86, P = 0.07$) |
| RV : LV mass ratio | $0.58M_b^{0.04 \pm 0.30}$ $r^2 = 0.01, P = 0.79$ | $0.32M_b^{0.02 \pm 0.15}$ $r^2 = 0.01, P = 0.80$ | $F_{1,17} = 0.018, P = 0.89$ ($F_{1,18} = 17.6, P < 0.001$) |

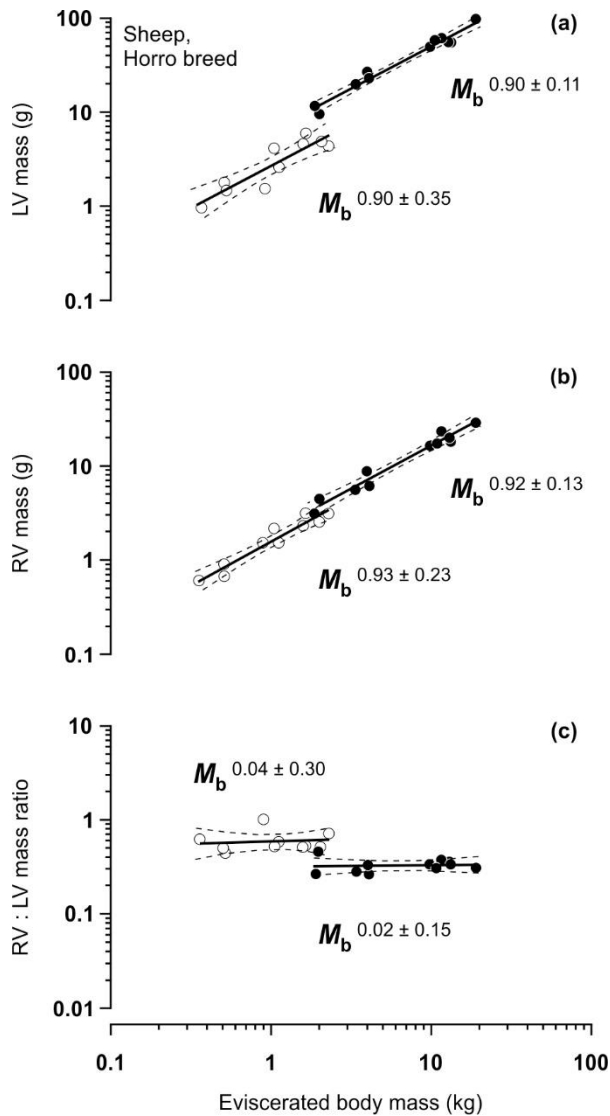
515 Equations are in the form $Y = aM_b^{b \pm 95\% CI}$, where Y is the cardiac variable of interest, a is the scaling coefficient
 516 (elevation), b is the scaling exponent (slope of the log-transformed relationship), M_b is eviscerated body mass in kg,
 517 and CI stands for confidence interval. LV is left ventricle and RV is right ventricle



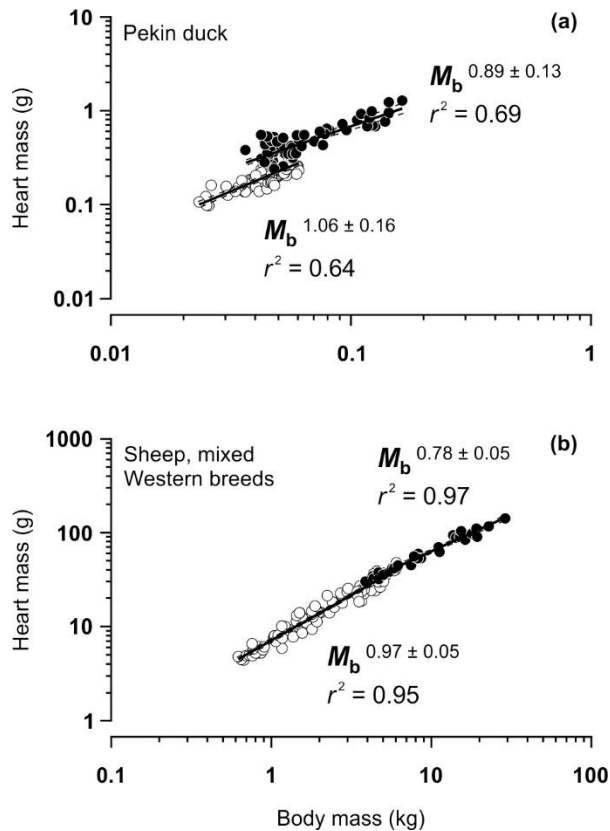
519
 520 **Fig 1.** (a) Schematic of the fetal heart showing the *foramen ovale* communication between the left and right atria,
 521 and the *ductus arteriosus* channel between the pulmonary artery and the aorta. These shunts close soon after birth
 522 in the normal neonatal heart. (b) Scaling as a tool to assess the fetal and postnatal heart as a function of body mass
 523 across development. A cardiac variable (Y) is plotted against body mass (M_b), to produce what is often a curvilinear
 524 relationship best defined by a power equation, $Y = aM_b^b$, where a (the coefficient) represents the elevation of the
 525 curvilinear line, and b (the exponent) describes the shape of the curvilinear line. The line is straightened for statistical
 526 analysis by log transformation, and the equation becomes, $\log Y = \log a + b \log M_b$, where b retains the same value but
 527 now defines the slope of the linearized relationship. The line also can be straightened, usually for graphical
 528 purposes, by plotting the arithmetic data on logged axes.



529
 530 **Fig 2.** (a) Scaling of whole heart mass (atria + ventricles) against eviscerated body mass (gastrointestinal tract
 531 removed) across the combined fetal and postnatal development of Horro sheep *Ovis aries* analysed in this study
 532 (~50-fold body mass range; $N = 21$). (b) Scaling of whole heart mass separated into fetal (unfilled circles; $N = 10$)
 533 and postnatal life stages (filled circles; $N = 11$). Broken stick analysis confirms breakpoint at birth. The exponents
 534 (slopes) are statistically indistinguishable between fetal and postnatal groups (ANCOVA, $P > 0.05$), but the elevations
 535 are significantly different ($P < 0.05$), because heart mass doubles around the time of birth. Solid line is the regression
 536 mean, dashed lines represent the 95% confidence band. Also presented for each relationship is the scaling exponent
 537 with 95% confidence interval. See Table 1 for complete scaling relationships and statistics.



538
 539 **Fig 3.** (a) Scaling of LV mass, (b) RV mass and (c) ventricular mass ratio (RV/LV) against eviscerated body mass in
 540 fetal (unfilled circles; $N = 10$) and postnatal (filled circles; $N = 11$) Horro sheep *Ovis aries*. Although the exponents
 541 (slopes) are statistically indistinguishable for LV mass and for RV mass when their respective fetal and postnatal
 542 groups are compared (ANCOVA, $P > 0.05$ for both LV and RV), there is a significant difference in elevation of the LV
 543 ($P < 0.05$), that is not apparent for the RV ($P > 0.05$), thus widening the ventricular mass ratio at birth. Solid line is
 544 the regression mean, dashed lines represent the 95% confidence band. Also presented for each relationship is the
 545 scaling exponent with 95% confidence interval. LV is left ventricle and RV is right ventricle.



546
 547 **Fig 4.** (a) Scaling of whole heart mass against yolk-free body mass in pre-hatch (unfilled circles; $N = 106$) and post-
 548 hatch (filled circles; $N = 92$) Pekin duck *Anas platyrhynchos domestica*, reproduced from previously published data
 549 (Sirsat et al., 2016). The exponents (slopes) are statistically indistinguishable between pre-hatch and post-hatch
 550 groups (ANCOVA, $P > 0.05$), but the elevations are significantly different ($P < 0.05$), because heart mass increases
 551 approximately 1.6-fold around the time of hatching. (b) Scaling of whole heart mass against body mass in fetal
 552 (unfilled circles; $N = 87$) and postnatal (filled circles; $N = 27$) sheep of mixed Western breeds *Ovis aries*, calculated
 553 from previously published data (Jonker et al., 2015). The exponents are significantly different between fetal and
 554 postnatal groups (ANCOVA, $P < 0.05$). Solid line is the regression mean, dashed lines represent the 95% confidence
 555 band. Also presented for each relationship is the scaling exponent with 95% confidence interval and the coefficient of
 556 determination.