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Echocardiographic reference ranges for sedentary Donkeys in the UK

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**Echocardiographic reference ranges for
sedentary Donkeys in the UK**

Manuscript prepared for *Veterinary Record*

By

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Echocardiographic reference ranges for sedentary Donkeys in the UK

Abstract

The aim of this study was to provide 2D and M-mode echocardiographic reference ranges from a sample of the UK population of donkeys including geriatrics (>30 years), owned by The Donkey Sanctuary, and to assess the influence of gender, weight and age on these variables. A total of 36 donkeys with no clinical or echocardiographic evidence of cardiovascular disease were examined; 24 geldings and 12 females, aged 3 – 45 years old, weighing 130 – 262 kg. Left atrial to aortic ratio was larger in geldings ($P=0.004$). There was no significant difference for left ventricular M-mode diastolic diameter between females and geldings ($P=0.121$) after exclusion of one heavy female outlier. 2D measurements significantly increased with body weight including maximal left atrial diameter ($R^2=0.112$; $P=0.046$), aortic diameter at various levels (e.g. annulus: $R^2=0.35$; $P<0.001$) and the pulmonary artery diameter ($R^2=0.124$; $P=0.035$). M-mode measurements were not significantly influenced by weight other than the left ventricular free-wall in systole ($R^2=0.118$; $P=0.041$). Age and heart rate did not have any significant effect on echocardiographic variables. This is the first UK study to report on echocardiographic reference ranges of sedentary donkeys across a wide age range, and shows differences compared to reference ranges from working donkeys.

Key words

Equus asinus, Cardiology, cardiac ultrasound, reference ranges

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44 Introduction

45 The total number of donkeys in the UK is estimated to be 10 000. Approximately 35% of these are
46 owned by The Donkey Sanctuary (Starkey and Starkey 1996) some of which are loaned out
47 (“adopted”) by independent carers (Cox and others 2010). Compared with the estimated 44 million
48 donkeys worldwide (Starkey and Starkey 1996), UK donkeys are not likely to be working (other than
49 some leisure donkeys) and their role is as a pet, companion or in animal assisted therapy for people
50 with special needs (Morrow and others 2011). Although the average age of working donkeys has
51 been reported as <15 years (Pritchard and others 2005), UK donkeys have been estimated to live at
52 least 20 years (Cox and others 2010) and at The Donkey Sanctuary, the average age of death in one
53 study was 30.5 years (Burden and others 2008).

54 Echocardiography is increasingly available as a non-invasive tool to screen for or diagnose cardiac
55 disease in all species. Echocardiographic studies of healthy donkeys have been published in the
56 literature to provide reference intervals, both for non-working (Amory and others 2004; Delvaux and
57 others 2001) and working animals (Hassan and Torad 2015). However numbers of animals and the
58 young age of donkeys included in these studies (n=20; 4 – 18 years (Amory and others 2004); n=12; 2
59 – 18 years (Delvaux and others 2001); n=30; 2 – 6 years (Hassan and Torad 2015) respectively) mean
60 that echocardiographic data regarding healthy geriatric donkeys and donkeys with cardiac disease
61 are lacking.

62 It is known in horses and humans that athletic cardiac remodelling can occur, identified by
63 echocardiography (Buhl and others 2005; Giada and others 1998; Young and others 2005), reversible
64 on de-training (Giada and others 1998; Kriz and others 2000), so it is unlikely that echocardiography
65 reference data from working donkeys can be applied to sedentary donkeys. Furthermore, ageing is
66 known to influence echocardiographic measurements in humans, even in the absence of systemic
67 hypertension or acquired cardiac disease, with smaller left ventricular (LV) lumen and thicker LV
68 walls (Gardin and others 1979). Indeed, normograms (Henry’s) are published for echocardiographic

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3 69 reference ranges in humans, taking into account their age (Feigenbaum 1994). The influence of
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5 70 ageing on echocardiographic measurements has not been explored in equidae, other than the age-
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7 71 related development of degenerative valve disease resulting in aortic regurgitation. Furthermore,
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9 72 horses do not live as long as donkeys, so donkeys may be more likely to demonstrate the effect of
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11 73 ageing on their echocardiographic variables.

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14 74 Therefore, the aim of this study was to provide 2D and M-mode echocardiographic reference ranges
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16 75 in donkeys with a wide age range, and to explore the effects of age, gender, body mass and body
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18 76 condition score on these echocardiographic variables.

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82 **Materials and Methods**

83 *Donkeys*

84 Donkeys in this observational cross-sectional study were selected from a population of 202 donkeys
85 at the Donkey Sanctuary which had undergone physical examination and auscultation (findings from
86 the whole groups detailed in parallel manuscript: Roberts & Dukes-McEwan). Donkeys were selected
87 from this population by a veterinary surgeon at The Donkey Sanctuary, from a wide range of ages, to
88 give a fairly even distribution within the age groups <15 years, 15 – 30 years and >30 years, located
89 on one farm. A further 6 donkeys were assessed by the author (SLR) at different locations at The
90 Donkey Sanctuary, under similar husbandry conditions. Donkeys were excluded from this
91 echocardiographic reference group if they had any audible heart murmur and any significant valvular
92 regurgitation noted on colour flow Doppler echocardiography (CFDE), affecting any valve (Roberts &
93 Dukes-McEwan). Other exclusion criteria were known cardiac disease or a record of previous
94 detection of a heart murmur, significant systemic disease which may affect the cardiovascular
95 system, skin disease affecting the right hemithorax which would preclude clipping for
96 echocardiography, and those donkeys non-compliant with handling. Chronic laminitis and dental
97 disease were not considered exclusion criteria.

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99 *2D and M-mode Echocardiography*

100 Donkeys had their right thoracic limb one stride forward (or held by a groom). Acoustic preparation
101 was achieved with clipping over the cardiac area on the right hemithorax, cleaning with hibitane and
102 wiping with surgical spirit, followed by liberal application of ultrasound gel. The investigator was
103 seated during the examination, on the right side of the donkey. Transthoracic echocardiography was
104 performed using an Esaote MyLab30Vet Gold ultrasound machine with a 2.5-3.5 MHz phased array
105 sector transducer. Standard cardiac 2D right parasternal long and short axis views were obtained in

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3 106 standing donkeys as previously described for horses (Marr and Patteson 2010). An
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5 107 electrocardiogram was recorded simultaneously to time cardiac events, with leads placed in a loose
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7 108 base apex configuration (electrodes were ECG pads, placed cranial to right scapular (red) and over
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9 109 left brisket (yellow) with the earth lead at the right thoracic inlet). The presence of structural heart
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11 110 disease and cardiac remodelling was assessed by 2D echocardiography. CFDE was used to exclude
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13 111 anything other than trivial or mild valvular regurgitation (Marr and Patteson 2010; Marr and Reef
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15 112 1995; Reef 1995). From short axis views of the left ventricle at chordae tendinae level, M-mode
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17 113 echocardiograms were obtained by placing the M-mode cursor to bisect the left ventricular cavity.
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19 114 Images were archived and subsequently reviewed and measured off-line. M-mode measurements
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21 115 followed standard protocol (Sahn and others 1978), using the leading edge to leading edge method
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23 116 (only including one proximal endocardium in each measurement). End-diastole was defined as the
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25 117 start of the QRS complex and end-systole as the nadir of septal motion. Two dimensional
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27 118 measurements at the heart base short axis, optimised for the aortic valve and left atrium, were
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29 119 obtained. From still frames during any point in diastole, usually early in diastole where the cusps of
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31 120 the closed aortic valve leaflets could be clearly visualised, the aortic diameter and left atrial diameter
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33 121 were measured, using inner edge - inner edge methods, as described for dogs (Hansson and others
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35 122 2002), using the line between left coronary and non-coronary cusps of the aortic valve to define the
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37 123 line of measurement for both aortic diameter and left atrial diameter (Figure 1A). From a right
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39 124 parasternal long axis 4 chamber view, with the left atrium optimised, the maximum left atrial
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41 125 diameter was measured at end-systole (frame before mitral valve opening) (Figure 1B). From the
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43 126 right parasternal long axis left ventricular outflow (5 chamber) view, aortic diameter was measured
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45 127 in systole at the level of the annulus, sinus of Valsalva and sino-tubular junction (Figure 1C). From a
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47 128 right parasternal cranial short axis view, optimised for the pulmonary trunk, the pulmonary annulus
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49 129 diameter was measured in systole.
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3 130 For each echo variable, at least three cardiac cycles, consecutive if possible, were measured and the
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5 131 mean values recorded. Cardiac cycles associated with arrhythmias (e.g. following 2nd degree
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7 132 atrioventricular block) were not included.
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10 133 *Statistical methods*
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13 134 Data were recorded in an Excel spreadsheet. Data were imported into SigmaPlot 13.0 for Windows
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15 135 (Systat software Inc.). Basic descriptive data are reported as means and standard deviation if data
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17 136 were normally distributed or as medians (minimum – maximum) if not. The Shapiro-Wilk test was
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19 137 used to assess for normality. Comparisons between males and females used the Student's unpaired
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21 138 two-tailed T-test for normally distributed data, or the Mann-Whitney test for non-normal
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23 139 distribution of data or where there was unequal variance. Although donkeys were selected to be
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25 140 from the three different age groups (<15 years, 15 – 30 years and >30 years), age was used as a
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27 141 continuous variable. Linear regression was used to explore associations between sets of variables,
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29 142 such as age and body weight, on echocardiographic data.
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33 143 A P value of <0.05 was accepted as representing statistical significance.
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3 147 **Results**
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5 148 A total of 53 donkeys underwent echocardiography. Four of these had heart murmurs and
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7 149 another 10 were identified on CFDE to have aortic insufficiency (Aoi) (Roberts & Dukes-
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10 150 McEwan), so were excluded from this echocardiographic reference population. One donkey
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12 151 was withdrawn because of dull demeanour and extensive skin disease. Another two were
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14 152 excluded because of incomplete echocardiographic studies. Three donkeys had chronic
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16 153 laminitis, included in the study, but none had other significant systemic disease. Therefore,
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18 154 a total of 36 donkeys underwent echocardiography, with a minimum of 10 in each age
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20 155 group (<15 years (N=13), 15 – 30 years (N=12) and >30 years (n=11)). All had normal
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22 156 echocardiograms based on 2D, M-mode and CFDE assessment.
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27 157 The description of the donkeys is shown in Table 1. There were 24 males, all geldings, and
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29 158 12 females. Donkeys were aged between 3 and 45 years old and weighed between 130 –
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31 159 262 kg. Heart rate documented during echocardiography was a median of 48.8 beats per
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33 160 minute (bpm) (range 38 – 70). There was no statistically significant difference between
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35 161 geldings and females in age, body weight or heart rate (Table 1). However, one female
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37 162 donkey, the largest in the study at 262 kg, 71 kg heavier than the next heaviest female (191
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39 163 kg) and 56 kg heavier than the next heaviest male (206 kg), was an outlier. If she was
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41 164 excluded, males (175.92 ± 21.33 kg) were significantly heavier than the majority of females
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43 165 (157.46 ± 20.80 kg) ($P=0.022$). Body condition scores were only available for 30/36 donkeys
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45 166 (Table 1), and was 3.0 in 24 of these donkeys, with 5 donkeys scoring 3.5 and one donkey
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47 167 4.5. Therefore, the effect of BCS was not explored further on the echocardiographic
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49 168 variables, given the lack of variance recorded.
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3 169 The M-mode echocardiography results for all the donkeys are shown in Table 2A. The only
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5 170 M-mode variable with a significant difference between genders was the LV diastolic
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7 171 diameter (LVDd), which was greater in females (Table 2B) (Figure 2). However, there was
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10 172 one outlying data point, with a 6 year old female, the largest donkey evaluated at 262 kg,
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12 173 having an 8.6 cm diameter left ventricle. If this donkey was excluded, the female mean (\pm
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14 174 SD) LVDd was 7.0 cm (\pm 0.5), not significantly different from the male LVDd (6.7 ± 0.6 cm) (P
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17 175 = 0.121).

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20 176 The 2D echocardiography results for all donkeys are shown in Table 3A. There was no
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22 177 difference between genders for short axis aortic diameter or long axis aortic diameters at
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24 178 valve, sinus of Valsalva or sino-tubular junction level (Table 3B). However, male donkeys had
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27 179 significantly greater short axis left atrial diameter than females which also similarly affected
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29 180 the short axis left atrial / aortic (LA/Ao) ratio (Table 3B).

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33 181 The effect of weight on echocardiographic variables was explored (Table 4). For M-mode
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35 182 variables, a statistically significant association between measurements and bodyweight was
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37 183 not evident, other than the systolic left ventricular free-wall thickness (Figure 3A). In
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39 184 contrast, however, all the 2D variables (long axis maximal left atrial diameter (systole), short
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41 185 axis left atrial diameter in diastole (Figure 3B), short axis diastolic aortic diameter, long axis
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43 186 aortic systolic diameter at valve annulus (Figure 3C), sinus of Valsalva and sinotubular
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45 187 junction levels and pulmonary artery annulus diameter (Figure 3D) showed significant weak-
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47 188 moderate positive associations with body weight (Table 4).

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53 189 Heart rate did not have any significant association with any of the M-mode or 2D variables.
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55 190 However, the 2D ratio of the aortic systolic diameter at valve level and the pulmonary artery
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3 191 diameter at valve level was weakly but significantly positively associated with heart rate

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5 192 (R=0.381; R² = 0.145; P=0.026).

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9 193 Despite a non-significant trend to increasing LV diastolic diameter and free wall thickness

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11 194 with advancing age, age did not show any statistically significant associations with any of the

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13 195 M-mode or 2D echocardiographic variables.

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197 **Discussion**

198 This is the first UK study to report on echocardiographic reference ranges from sedentary
199 donkeys with a wide age range, including a number of geriatric donkeys. Echocardiographic
200 variables however, show very similar results to those shown in other studies of sedentary
201 donkeys (Amory and others 2004; Delvaux and others 2001). Furthermore, in this study, we
202 did not demonstrate any significant association with age on any of the echocardiographic
203 variables, in contrast to data from humans. Although not statistically significant, there were
204 trends to increasing LV chamber diameter in diastole and systole with age in this study,
205 which is converse to that reported in humans, who have smaller chambers and thicker walls
206 with advancing age (Feigenbaum 1994; Kou and others 2014). There is little information in
207 the veterinary literature about the effect of ageing on echocardiographic dimensions in any
208 species, although wall thickness increases in Cavalier King Charles spaniels without a
209 significant change in LV chamber diameter (Misbach and others 2014). Despite the wide age
210 range of donkeys in this study, these data suggest that age does not have to be taken into
211 account when comparing echocardiographic values with reference values.

212 As in horses and other species, body size should be taken into account in assessing
213 echocardiographic data with reference ranges. From our data, however, there were no
214 significant associations with body weight on most of the M-mode variables. This is in
215 contrast to significant linear regression associations shown previously in donkeys (Amory
216 and others 2004), including working donkeys (Hassan and Torad 2015). In the study by
217 Amory and others (2004), there was a wider weight range (98 – 255 kg) than this study,
218 which may explain the discordant finding, but the weight range was slightly more narrow in
219 the Hassan and Torad (2015) study (150 – 220 kg) than ours (130 – 262 kg). Even though the

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3 220 number of donkeys included in our study is higher than the other studies, the linear
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5 221 regression analyses were statistically under-powered, which means a genuine influence of
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7 222 body weight on M-mode variables was simply not detected. The lack of a significant
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10 223 influence of body weight on M-mode echocardiographic data is an advantage in the field,
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12 224 however, as weighing scales may not be available at most premises. We did demonstrate
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14 225 significant positive associations with 2D measured variables and body weight, including the
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16 226 left atrium, aortic diameters at the various levels and the pulmonary artery diameter.

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20 227 These data show that gender does not need to be taken into account in assessing most
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22 228 echocardiographic measurements, at least between geldings and females, as jacks (entire
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24 229 males) were not included. There was no statistically significant difference in body weights
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26 230 between the two genders in this population. However, there was a heavy, outlying female
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28 231 donkey, which, if excluded, resulted in males being significantly heavier. Indeed, there were
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30 232 no significant gender population differences or for most echocardiographic variables which
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32 233 is why echocardiographic data were combined for both genders. In our study, the left
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34 234 atrium (short axis diastolic) diameter was significantly greater in geldings than females,
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36 235 which also affected the LA/Ao ratio. However, this is not likely to be of clinical importance.
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38 236 In humans, the larger left atrium in males than females is no longer evident after
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40 237 normalising for body surface area (Kou and others 2014).

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44 238 The echocardiographic data generated in this study, although similar to other data from
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46 239 sedentary donkeys (Amory and others 2004; Delvaux and others 2001), show some marked
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48 240 differences compared with data generated in another study of 30 working donkeys (Hassan
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50 241 and Torad 2015). Those donkeys were of similar weight range to those reported here, but
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52 242 the mean M-mode diameters of the LV in diastole (LVDd) and systole (LVDs) are markedly
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3 243 smaller (LVDD range: 3.84 – 5.83 cm; LVDs range: 2.13 – 3.44 cm) than this study (LVDD: 5.4
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5 244 – 8.6 cm; LVDs: 2.9 – 5.1 cm). As LV wall thickness in diastole and systole from both studies
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7 245 were similar, the relative wall thickness (LVFWd /LVDD ratio) is higher in the working
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9 246 donkeys, calculated from the mean values (1.42/4.82) as 0.29, than the sedentary donkeys
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11 247 in this study (1.7/6.8), at 0.25. This could represent the effect of work and physiological
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13 248 remodelling. However, methodological differences between the two studies is also possible,
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15 249 The figure showing M-mode acquisition in the study of Hassan and Torad (2015) suggests a
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17 250 more apical level of the LV than chordal level, which may result in the smaller LV cavity.
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19 251 Furthermore, in healthy horses of a range of body sizes, level of work reported by owners
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21 252 did not appear to significantly influence echocardiographic results (Al-Haidar and others
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23 253 2013), although other studies do demonstrate remodelling changes in horses with training
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25 254 (Buhl and others 2005; Young 1999), but with increases in the LV chamber dimensions,
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27 255 rather than reduction. This may reflect the different work that donkeys do, compared with
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29 256 horses. More information is required on the effect of work (and type of work) on the
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31 257 echocardiographic variables in the donkey. The effect of breed of donkey should also be
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33 258 investigated in future work, not investigated in our study of Standard donkeys. A
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35 259 homogenous breed (Baladi Egyptian donkeys) were assessed by Hassan and Torad (2015).
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44 260 Allometric scaling from body weight is increasingly advocated in the generation of
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46 261 echocardiographic reference ranges (Cornell and others 2004), and this has been proposed
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48 262 for horses of a wide range of body weights (Al-Haidar and others 2013). The donkeys in this
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50 263 study had measured and calculated measurements for LVDD and LVDs which were not
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52 264 significantly different between actual and calculated measurements, but they did not
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54 265 correlate and there were very wide limits of agreement on Bland-Altman plots (data not
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3 266 shown). Future work to generate data from donkey (possibly donkey breed) specific
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5 267 allometric scaling reference ranges based on weight or body size would be useful to refine
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8 268 echocardiographic reference ranges. Indexing chamber sizes to aortic diameter has also
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10 269 been advocated to normalise for patient size, which shows similar results across species
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12 270 (cats, dogs, horses) (Brown and others 2003). This approach may also applied to donkeys in
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15 271 future work.

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18 272 There are limitations of this study. Although 36 donkeys were included in the study, some
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20 273 statistical methods were under-powered, so larger numbers would have strengthened the
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22 274 data from this study. Due to time constraints, and reluctance to impose further on The
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24 275 Donkey Sanctuary staff, we did not repeat echocardiograms, so intra- and inter-observer
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27 276 echocardiographic repeatability was not assessed, although this is important to know before
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29 277 inferring changes with serial echocardiography, or in interpreting deviations from normal
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31 278 data in an individual case. Only standard M-mode and 2D measurements were obtained for
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33 279 this study; we did not generate reference ranges for spectral Doppler, which may also be
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36 280 important in investigating the effects of ageing or disease. Finally, as body condition scores
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38 281 were very similar in the population, with only one over-weight and no poor-condition
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41 282 donkeys, the influence of BCS on echocardiographic variables could not be assessed.

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3 284 **Conclusions**
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6 285 Standard M-mode and 2D echocardiographic reference ranges generated from 36 UK
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8 286 donkeys of wide age range are similar to those reported for other sedentary donkeys. Body
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10 287 weight did not significantly affect M-mode variables, but did influence 2D variables,
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12 288 particularly of aortic and pulmonary artery diameters, and this should be considered,
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14 289 particularly with very small or very large donkeys. This study showed that as age, gender
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16 290 and heart rate did not have any important influence on echocardiographic variables, they do
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18 291 not need to be considered when comparing patient data to these reference values.
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301 Donkey Sanctuary for her constructive criticism of this manuscript.

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TABLES

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Table 1: Population description (N=36)

	Mean \pm SD (or median)	Confidence interval of the mean	Minimum - Maximum	
Gender				24 G; 12F
Weight (kg)	172.67 \pm 27.0	9.15	130 - 262	
BCS (/5) (n=30)	Median: 3	NA	3.0 – 4.5	
Age (years)	20.58 \pm 11.9	4.02	3 - 45	
Heart rate (bpm)	48.80 \pm 7.5	2.54	38 - 70	
				Differences between genders (P value)
Geldings Weight (kg)	Median: 173.5	NA	138 – 215	
Females Weight (kg)	Median: 163	NA	130 - 262	P = 0.072
Geldings BCS (/5)(n=21/24)	Median: 3	NA	3 – 4.5	
Females BCS (/5)(n=9/12)	Median: 3	NA	3 - 3.5	P=0.435
Geldings Age (years)	19.0 \pm 12.5	5.26	3 – 45	
Females Age (years)	23.75 \pm 10.3	6.57	6 – 36	P=0.264
Geldings heart rate (bpm)	50.04 \pm 8.3	3.5	41 - 70	
Females heart rate (bpm)	46.5 \pm 5.4	3.4	40 - 56	P=0.189

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308 Abbreviations: BCS; body condition score, bpm, beats per minute, NA not applicable.

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Table 2A: M-mode echocardiography results from 36 donkeys

	Mean \pm SD	Confidence interval (CI) of the mean	Minimum	Maximum
RVDd (mm)	20.7 \pm 3.9	1.3	13.5	30.4
IVSd (mm)	16.2 \pm 2.4	0.80	10.7	22.4
LVDd (mm)	68.1 \pm 6.7	2.3	54.0	85.7
LVFWd (mm)	17.0 \pm 2.6	0.9	11.1	23.2
IVSs (mm)	25.8 \pm 3.5	1.2	18.8	33.4
LVDs (mm)	40.7 \pm 5.2	1.8	29.3	51.2
LVFWs (mm)	22.7 \pm 3.6	0.6	15.4	30.3
FS (%)	39.7 \pm 7.3	1.2	27.0	56

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Table 2B: M-mode echocardiography results: Males (N=24) and Females (N=12) separated

	Mean \pm SD (males)	CI of the mean (males)	Mean \pm SD (females)	CI of the mean (females)	P value
RVDd (mm)	21.8 \pm 4.0	1.7	18.5 \pm 2.8	1.8	0.140
IVSd (mm)	16.4 \pm 2.3	1.0	15.7 \pm 2.6	1.6	0.458
LVDd (mm)	66.5 \pm 6.3	2.7	71.3 \pm 6.6	4.2	0.022
LVFWd (mm)	16.8 \pm 2.6	1.1	17.4 \pm 2.8	1.8	0.480
IVSs (mm)	25.5 \pm 3.6	1.4	26.4 \pm 3.9	2.5	0.461
LVDs (mm)	40.3 \pm 5.0	2.1	41.5 \pm 5.8	3.7	0.542
LVFWs (mm)	23.0 \pm 3.6	1.3	22.2 \pm 3.50	2.2	0.521
FS (%)	39.0 \pm 7.1	3.0	41.0 \pm 7.6	4.8	0.460

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3 313 Significant differences between genders are indicated in bold.
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5 314 Abbreviations: SD: standard deviation, RVDd: Right ventricular diameter in Diastole, IVSd:
6 315 Interventricular septum thickness in diastole, LVDd: left ventricular diameter in diastole, LVFWd: Left
7 316 ventricular free-wall thickness in diastole, IVSs: Interventricular septum thickness in systole, LVDs:
8 317 left ventricular diameter in systole, LVFWs: Left ventricular free-wall thickness in systole, FS:
9 318 fractional shortening.
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Table 3A: 2D echocardiography results from 36 donkeys

	Mean \pm SD	Confidence interval (CI) of the mean	Minimum	Maximum
LA Long axis maximal diameter (systole) (mm)	63.7 \pm 6.3	1.1	46.8	74.3
LA diameter d (short axis) (mm) (n=34)	54.0 \pm 5.2	1.8	43.7	63.4
Ao diameter d (short axis) (mm) (n=34)	40.8 \pm 4.3	1.5	32.9	48.7
LA/Ao ratio (n=34)	1.32 \pm 0.1	0.05	1.08	1.65
Ao diameter valve s (long axis) (mm)	35.6 \pm 3.9	1.3	29.5	43.2
Ao diameter s, sinus of Valsalva (mm)	42.0 \pm 4.5	1.5	33.2	52.5
Ao diameter s, sinotubular junction (mm)	35.7 \pm 3.8	1.3	28.4	43.6
PA diameter (mm)	32.2 \pm 4.10	1.4	25.8	43.3
Ao/PA ratio	1.27 \pm 0.16	0.05	0.89	1.5

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Table 3B: 2D echocardiography results: Males (N=24) and Females (N=12)

	Mean \pm SD (males)	CI of the mean (males)	Mean \pm SD (females)	CI of the mean (females)	P value
LA Long axis maximal diameter (systole) (mm)	64.6 \pm 5.5	2.3	61.8 \pm 7.6	4.9	0.204
LA diameter d (short axis) (mm) (n=34)	55.9 \pm 4.4	1.9	50.6 \pm 5.0	3.2	0.004
Ao diameter d (short axis) (mm) (n=34)	41.0 \pm 4.4	1.9	40.4 \pm 4.5	2.8	0.684
LA/Ao ratio (n=34)	1.36 \pm 0.13	0.06	1.25 \pm 0.09	0.06	0.015
Ao diameter valve s (long axis) (mm)	35.5 \pm 3.8	1.6	35.6 \pm 4.2	2.7	0.947
Ao diameter s, sinus of Valsalva (mm)	42.0 \pm 4.6	1.9	42.2 \pm 4.4	2.8	0.874
Ao diameter s, sinotubular junction (mm)	35.42 \pm 3.50	1.5	36.4 \pm 4.3	2.7	0.475
PA diameter (mm)	32.36 \pm 4.18	1.8	31.9 \pm 4.1	2.6	0.749
Ao/PA ratio	1.27 \pm 0.18	0.07	1.23 \pm 0.18	0.07	0.450

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324 Significant differences between genders are indicated in bold.

325 Abbreviations: SD: standard deviation, LA: left atrium, Ao: aorta, d: diastole, s: systole, PA:

326 pulmonary artery (annulus), mm: millimetres.

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Table 4: Association of Body Weight on Echocardiographic variables

	R	R ²	P	Linear regression equation for significant associations
M-mode variables				
RVDd			0.201	
IVSd			0.908	
LVDd			0.075	
LVFWd			0.051	
IVSs			0.139	
LVDs			0.161	
LVFWs	0.343	0.118	0.041	LVFWs = 14.94 + (0.045 x Weight Kg)
FS			0.680	
2D variables				
LA s max	0.334	0.112	0.046	LA LX s = 50.242 + (0.0780 x Weight Kg)
LA d	0.474	0.225	0.005	LAd d = 38.739 + (0.0886 x Weight Kg)
Ao d	0.417	0.174	0.014	Ao d = 29.566 + (0.0651 x Weight Kg)
Ao valve s	0.579	0.35	<0.001	Ao valve d = 21.324 + (0.0825 x Weight Kg)
Ao S of V s	0.495	0.245	0.002	Ao S of V d = 27.939 + (0.0816 x Weight Kg)
Ao sinotub s	0.558	0.312	<0.001	Ao ST junc d = 22.370 + (0.0774 x Weight Kg)
PA	0.352	0.124	0.035	PA = 22.974 + (0.0534 x Weight Kg)

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3 329 Significant associations are indicated in bold, and relevant significant linear regression equations are
4 330 only provided for significant associations.
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6 331 Abbreviations: For M-mode abbreviations, see Table 1. LA: left atrium (maximal diameter, long axis 4
7 332 chamber view), s: systole, LA d: Left atrium (short axis) diastole, Ao d: Aortic diameter (short axis,
8 333 level of aortic valves), diastole, Ao valve s: Long axis aortic annulus diameter at valve insertion level,
9 334 systole, Ao S of V s: aortic diameter (long axis) in sinus of Valsalva, systole, Ao sinotub s: aortic
10 335 diameter at junction of sinus of Valsalva and tubular aorta (long axis), systole. PA: Pulmonary artery
11 336 annulus diameter (during systole).
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3 342 **Figure legends**

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7 344 **Figure 1: Illustrations of 2D measurements made with electronic callipers on**
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10 345 **echocardiographic images.**

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13 346 A. 2D right parasternal short axis view at level of the aortic valves, with optimization
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15 347 of the left atrial size. The aortic valves can be seen, closed early in diastole. The
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17 348 aortic diameter measurement is made following the line of the non-coronary and
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20 349 left coronary cusps, and left atrial measurement is also shown, following this line.

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22 350 B. 2D right parasternal long axis view, with optimization of the left atrial diameter.
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25 351 Maximal left atrial diameter is measured at the end of ventricular systole, before
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27 352 the mitral valve leaflets open.

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29 353 C. 2D right parasternal long axis view, showing left ventricular outflow and the
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31 354 aorta. This is during ventricular systole, when the aortic valves are open.
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33 355 Measurements were made at the aortic annulus level, sinus of Valsalva, and sino-
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35 356 tubular junction. (A small part of the left atrium was also measured in the image,
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37 357 but data not used in this study).

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44 359 **Figure 2: Box and Whisker plot showing the M-mode left ventricular diastolic diameter**
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46 360 **between Gelding and Female Donkeys. Box defines 25 and 75 percentile, whiskers 10 and 90**
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48 361 **percentile, with outliers indicated by dots. Median line shown within the box.**

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3 363 **Figure 3:** Associations between body weight and echocardiographic variables by linear
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5 364 regression analyses (see also Table 4). The regression line is shown, with 95% confidence
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7 365 intervals for the line, and 95% prediction intervals for the data.
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11 366 Figure 3A: M-mode LV free wall in systole and body weight
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13 367 Figure 3B: 2D LA diameter (diastole) (short axis view) and body weight
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15 368 Figure 3C: 2D aortic diameter at annulus level (long axis) (systole) and body weight
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17 369 Figure 3D: 2D pulmonic annulus diameter (systole) and body weight
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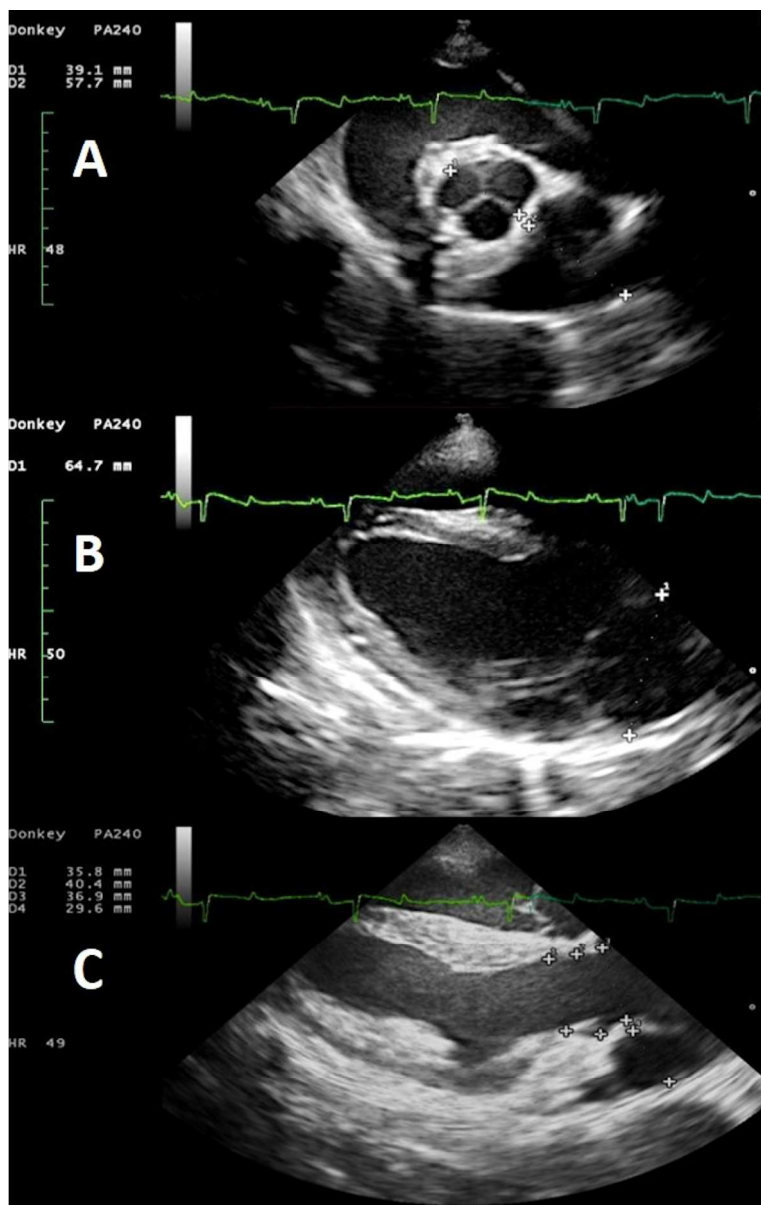


Figure 1: Illustrations of 2D measurements made with electronic callipers on echocardiographic images.

A. 2D right parasternal short axis view at level of the aortic valves, with optimization of the left atrial size. The aortic valves can be seen, closed early in diastole. The aortic diameter measurement is made following the line of the non-coronary and left coronary cusps, and left atrial measurement is also shown, following this line.

B. 2D right parasternal long axis view, with optimization of the left atrial diameter. Maximal left atrial diameter is measured at the end of ventricular systole, before the mitral valve leaflets open.

C. 2D right parasternal long axis view, showing left ventricular outflow and the aorta. This is during ventricular systole, when the aortic valves are open. Measurements were made at the aortic annulus level, sinus of Valsalva, and sino-tubular junction. (A small part of the left atrium was also measured in the image, but data not used in this study).

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Left ventricular M-mode diameter, diastole

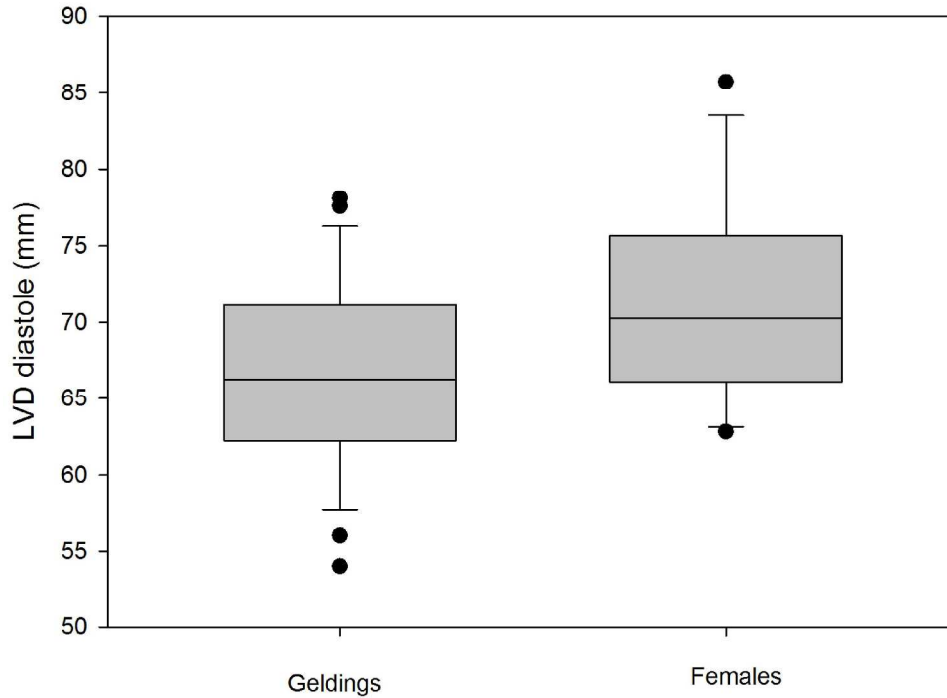


Figure 2: Box and Whisker plot showing the M-mode left ventricular diastolic diameter between Gelding and Female Donkeys. Box defines 25 and 75 percentile, whiskers 10 and 90 percentile, with outliers indicated by dots. Median line shown within the box.

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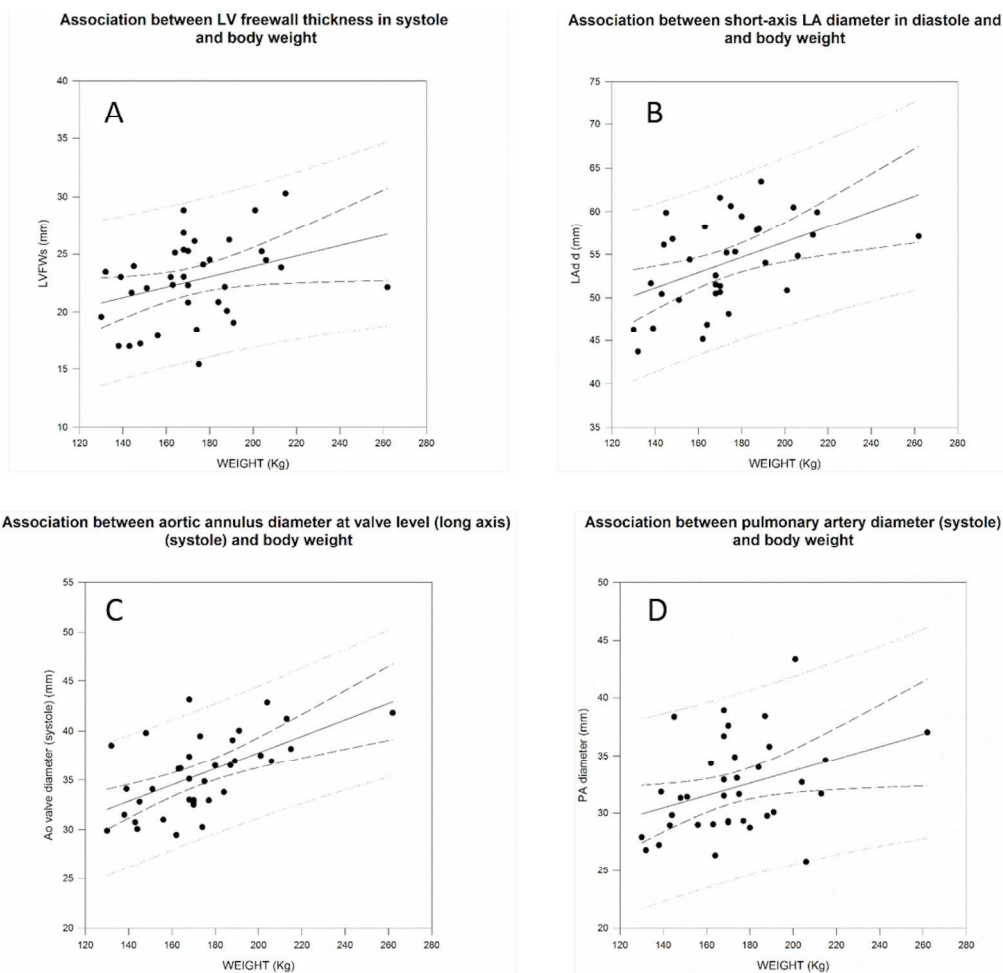


Figure 3: Associations between body weight and echocardiographic variables by linear regression analyses (see also Table 4). The regression line is shown, with 95% confidence intervals for the line, and 95% prediction intervals for the data. Figure 3A: M-mode LV free wall in systole and body weight. Figure 3B: 2D LA diameter (diastole) (short axis view) and body weight. Figure 3C: 2D aortic diameter at annulus level (long axis) (systole) and body weight. Figure 3D: 2D pulmonic annulus diameter (systole) and body weight.

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