Heart performance of lambs and its relation to muscle volume and body surface

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8   **Abstract**

9   ECG gated dynamic magnetic resonance imaging (MRI) methodology was developed for in

10   vivo examination of the sheep heart characteristics combining non- invasive determination

11   of skeletal muscle mass, to study the relationship between total body skeletal muscle

12   content and heart performance. Measurements were carried out in merino type male lambs

13   using a 1.5 T field-strength equipment. During post processing of the images average left

14   ventricular volumes were determined and stroke volume (SV) was estimated. Ejection

15   fraction was calculated ($73 \pm 1.8\%$) and the cardiac output (CO) value was estimated (2.75

16   ± 0.16 L/min). After measuring left ventricular wall thickness, contraction values were

17   determined at the septum (62%), at the anterior (69%), the lateral (54 %) and the posterior

18   (58%) walls and the ventricular mass were calculated. Immediately after the MRI

19   examination, body composition measurement was performed by computerized tomography

20   (CT) during the same narcosis. From the interpretation of the functional MRI and

volumetric CT results, relative CO value was developed, expressing the relationship between heart performance and total body skeletal muscle volume. Finally, CO value related to body surface was estimated (18.3 ± 3.1 dm^2/(L $*$ min^{-1})) to characterize the metabolic rate.

Key words: Lamb, Magnetic Resonance Imaging, Computerized Tomography, Cardiac performance

1. Introduction

The use of electromagnetic waves is gaining more and more space in different areas of animal sciences from meat quality determination (1,2) to in vivo evaluation of body composition applied as a possible mean for genetic improvement of a wide variety of species (3-5).

Moreover, using animal models (mouse, rat, rabbit, canine, swine, and sheep) in human cardiac research give invaluable opportunity to develop new diagnostic and therapeutic methods (6,7).

Magnetic resonance imaging (MRI) was firstly utilized as an in vivo technique for the determination of body composition of animals by Kallweit et al. (1994). A study in sheep indicates that the quantification of lean and fat tissue by means of prediction equations is highly accurate (9). The description of the ECG-gated MRI for the in vivo measurement of heart performance in pigs has been presented by Petrási et al. (2001), and an ovine medical model was first published by Pilla et al. (2003). Recently the sheep is used as a model of

45   cardiac MR imaging of different heart failures such dilated cardiomyopathy based on

46   T1mapping sequence (12). In an ovine model end-systolic volume were measured after

47   myocardial infarction with and without micro pump based partial mechanical circulatory

48   support using MRI method (13). The cardiac MRI method, together with computerized

49   tomography (CT) was applied to measure the functional heart performance of the pig and

50   heavy type turkey, in connection with body tissue composition determination (14,15). In

51   addition, as a further quantitative method for the determination of ovine cardiac

52   characteristics the real-time 3D echocardiography, as applied by Schmidt et al. (2001), also

53   may provide opportunity (16). Moreover, Segers et al. (2001) developed an indirect

54   prediction method for the heart performance characterization of sheep (17).

55   The results achieved in intensive pigs and turkeys indicate disadvantageous changes in

56   heart performance caused by the continuous selection for increasing skeletal muscle

57   volume. From this aspect the sheep seems to be less affected at present however the

58   situation may change in the future if selection for increased muscle building capacity

59   continues.

60

61   The goal of this experiment was to develop an appropriate in vivo method for the

62   quantitative measurement of the sheep heart performance and to determine basic heart

63   performance data jointly with skeletal muscle volume and body surface estimation.

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65   **2. Materials and methods**

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67   Methodological measurements were carried out on three merino type male lambs at the live

68   weight of 20 kg (60 ± 4 days of age). Investigations were performed at the Health Center of

69   Kaposvár University using its Siemens Magnetom Vision Plus type, magnetic resonance

70   tomograph equipment of 1.5 T magnetic field-strength and a Siemens Somatom S40 spiral

71 CT scanner. The lambs were pre medicated intramuscularly with Rometar (Xylazin 2%)

72 (Spofa; 0.2 mg/body weight kg). Following inhalation anesthesia was introduced through a

73 narcotic mask, using 3 vol% Isoflurane (Abbott Lab.) until reaching the total relaxation,

74 when the animals were intubated, and attached to a narcotic unit (Penlon evaporator,

75 Ohmeda flow-meter). Continuous deep narcosis was obtained using 1.5-2 vol% Isoflurane

76 and 2 vol% oxygen as carrier gas, similarly to the recommendation of Hikasa et al. (2000).

77

78 The MR examination was conducted using ECG-gated sequences (19). To obtain the proper

79 signal strength from the electrocardiograph, a special active electrode (Bruker Medical) was

80 used. The electrode attached to the signal cable was fixed 10 cm from the sternum, left,

81 between the third and sixth ribs, the other two directed to the left olecranon between the

82 third and sixth ribs. During the imaging process the animals were placed into a MR

83 compatible special plastic container into ventral position with extended limbs and were

84 fixed with belts. At first, quick images were taken to locate the heart according to the co-

85 ordinate system of the body. Following in the sagittal, coronal and transversal planes

86 localization images were taken to allocate the longitudinal axis of the heart. Then multislice

87 - multiphase images were taken orthogonal to the longitudinal axis of the heart from the

88 apex to the base, performing prospective data acquisition (10). The total data acquisition

89 took ca. 10-12 minutes. Altogether 9 slices and in each slice 9 images (phase) were

90 acquired according to one heart cycle (singleslice - multiphase). From the 9 transversal

91 slices representing the total heart volume 6 covered the ventricles. The phases applied were

92 characterized by the following data: echo time: 6.8 ms, repetition time: 60.0 ms, θ: 30º,

93 field of view: 400-500 mm, matrix size: 256×256 pixels, slice thickness: 8 mm, slice gap: 1

94   mm. Moreover, the left ventricular volumes were measured and the stroke volume (SV)

95   was also computed as the difference between the end-diastolic and end-systolic data. The

96   calculated ejection fraction (EF) is given as the percentage of the ratio of the SV to end-

97   diastolic volume. The left ventricular mass was calculated from the regions epicardium –

98   endocardium + papillary 1 + papillary 2 (marked in Figure 3.) times 1.05 (g/cm³). The

99   cardiac output (CO) value was estimated as a product of the SV and heart rate. The wall

100   thickness was measured by the septum, and by the anterior, lateral and posterior walls, 9

101   mm beneath the atrioventricular valve. Images were evaluated using the software MASS

102   4.1 (20).

103

104   Serial CT images were taken continuously from the neck (atlas) until the hock with 10 mm

105   slice thickness. The total body skeletal muscle volume and the body surface were

106   measured. The image analysis performed is described in detail by Romvári et al. (2004).

107

108   **3. Results**

109

110   The heart rate was monitored before and during the MRI examination. The pulse registered

111   during the total time was that according to the relaxed state (89±3 b/min). The

112   electrocardiogram attained from the foregoing time of examination gave accurate

113   information about the overall conditions and the anesthetic possibilities concerning each

114   animal.

115 The MR examination was conducted using ECG-gated sequences. Dynamic images were

116 taken orthogonal to the longitudinal heart axis, leading from the apex to the base, covering

117 all ventricles and atria, performing prospective data acquisition (Figure 1).

118 *Figure 1*

119

120 In Figure 2 six multislice-multiphase images are shown between the end diastolic- and the

121 end systolic phase.

122 *Figure 2*

123 During the post processing of the MR images the contour of the ventricular epicardium and

124 endocardium, the outline of the left ventricular papillaries was defined (Figure 3).

Figure 3 125

126 The changes of volumetric and wall thickness data of the heart cycle in time are depicted in

127 Figure 4.

Figure 4 128

129 The first phase is synchronic with the ECG R-wave, being the beginning of the isometric

130 contraction. Ventricular volume is at the maximum at this point. As the ventricular diastole

131 ends (5th to 6th phase), the phase of isometric relaxation begins. From methodological

132 point of view to characterize the heart function, it is not necessary to follow the whole heart

133 cycle, covering the phase from the end-diastole to the end-systole is sufficient.

134

135 Characteristic data concerning lamb heart performance shown in Table 1 are mean values

136 determined during the heart cycle.

137

138   Immediately after the MRI examination of the lambs, CT scanning was performed during

139   the same narcosis. First the total body muscle volume, than the body surface was measured

140   from the consecutive cross-sectional images. Resulting from the joint interpretation of MRI

141   and CT data the CO related to body surface value ($dm^2 / (L * min^{-1})$) was estimated and the

142   so called relative CO value (expressing the relationship between heart performance and

143   skeletal muscle volume in sedentary conditions ($dm^3 / (L * min^{-1})$)) was developed (Table 2).

Table 2
144

145   **4. Discussion**

146

147   When comparing our results with available data from the literature results obtained by

148   echocardiography also have to be considered taking the paucity of such results into account.

149   However it must be stressed that no echocardiography based cardiac data of sheep with

150   normal physiological state are available in the literature. The accessible data concerning in

151   vivo model arise from experiments aiming medical purposes where surgically operated

152   animals were used. Findings of Qin et al. (2000) are originated exactly from that kind of

153   experiment. They found that the LVEDV, LVESV and LVSV values were 65 ± 24, 30 ± 11

154   and 35 ± 11 ml respectively in case of sheep's with an average body weight of 40 kg (21).

155   It was concluded also by them that the accuracy of the method can be verified by cardiac

156   MR imaging as the gold standard method. Slightly higher LVESV (37.1 ± 8.8 ml) was

157   measured and a CO value of 3.8 L/min (± 0.6) was estimated by Psaltis et al. (2008)

158   applying cardiac MRI on merino sheep (51 ± 8.1 kg) (22). These results are in good

159   accordance with our data taken the live weight difference (20 vs. 40 kg) into account.

160

161   The average 73 % ejection fraction value is remarkably higher than the corresponding data

162   of the Mangalica pig (57%), the intensive meat type pig (53%) (14) or the giant turkey

163   (51%) (15). Lower ejection fraction refers to unfavorable hemodynamic characteristics of

164   the heart. The contraction values (septal, anterior, lateral and posterior: 62%, 69%, 54% and

165   58%, respectively) calculated from the measured wall thickness data are characteristic to

166   the condition of the myocardium. Cardiomyopathy or local circulatory failures cause a

167   functional anomaly of muscle fibers, and decrease the contraction values. The deviation

168   from normal in case of the ventricular volume and contraction data is of high diagnostic

169   value.

170

171   The average heart rate of 89 bpm can be interpreted as a normal physiological condition

172   (23). The estimated CO value – arising of the stroke volume and heart rate - is one of the

173   most important measures of heart performance in human medical practice. In a sedentary

174   state, some 16 % of the cardiac output is needed to supply the skeletal musculature in

175   humans (24). According to Meyns el al. (2000), the perfusion of the sheep muscle is 6 ± 3

176   ml/g of tissue (25). Considering the measured average of 6.4 dm^3 muscle tissue and the

177   estimated 2.75 L/min CO value, altogether 14% of the total CO supplies the musculature of

178   the examined lambs under narcosis. The estimated CO value is in good accordance with the

179   corresponding data (3.4 L/min) of Geens et al. (13). Cardiac output redistribution was

180   studied in a basic experiment carried out by Hales et al. (1984) on a merino model (26).

181   According the authors the competition between skin and muscle for blood flow during

182   exercise results a lower skin perfusion which could be critical in case of heat stress.

183

184   In the human medicine the cardiac output value related to body surface is widely used to

185   characterize the metabolic rate. In our study the respective data were 18.4 $dm^2 / (L * min^{-1})$

186   which is considerably lower than the corresponding value of the BUT Big 6 turkey at the

187   same 20 kg live weight (15).

188

189   The developed "relative cardiac output value" expresses the relationship between the lamb

190   heart performance and skeletal muscle volume in sedentary conditions. In general, the

191   physical load and/or stress-induced movements of animals lead to significant changes of

192   their blood flow distribution. An example of this phenomenon was studied by Animut and

193   Chandler (1996) in ewes describing a 25 % decrease on the mammary blood flow during

194   treadmill exercise (27). In contrast, Segers et al. (2001) developed a purely mathematical

195   model, for the estimation of stroke volume during strongly differing hemodynamic

196   conditions in sheep. At maximum O_2 consumption, 87 % of the cardiac output supplies the

197   skeletal muscles in miniature pigs (28). The measured average relative cardiac output

198   values (2.3 dm^3/(L $*$ min^{-1}) are similar to the corresponding data of the native slow growing

199   Mangalica pig (2.8 dm^3 / (L $*$ min^{-1}) at 30 kg live weight) (14). This favorably low value

200   refers to a well-balanced cardiovascular system characteristic for this semi-intensive sheep

201   breed.

202

203   A dynamic MR imaging protocol of the sheep heart was developed in our methodological

204   experiment. The preconditioning, the specific details of ECG measurement and MR

205   imaging were elaborated. The method combined with CT imaging process of the total body

206   gives the unique opportunity to study the skeletal musculature quantitatively together with

207   the heart performance in a non-invasive manner.

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Table 1   Some characteristic data concerning lamb heart performance

	LVEDV ml	LVESV ml	LVSV ml	LVEF %	LVMass g	HR b/min	CO L/min	Septum mm	Anterior mm	Lateral mm	Posterior mm
Average	43	11.2	31.8	77.9	63.5	89	2.75	13.6	11.7	11.7	14.2
SD	5.6	1.8	3.8	1.8	5.6	12	0.16	0.0	0.1	0.3	0.1

Where: LVEDV = left ventricular end-diastolic volume, LVESV = left ventricular end-systolic volume, LVSV = left ventricular stroke volume, LVEF = left ventricular ejection fraction, LVMass = left ventricular mass, HR = heart rate, CO = cardiac output

Table 2   Related cardiac output data

	Muscle volume (dm³)	Body surface (BS) (dm²)	CO related to BS $dm^2 / (L * min^{-1})$	Relative CO $dm^3 / (L * min^{-1})$
Average	6.4	50.3	18.3	2.3
SD	0.79	5.5	3.1	0.26