Echocardiographic findings in apparently healthy Czechoslovakian wolfdogs

Ivasovic, Frane ; Poletti, Giovanni ; Baron Toaldo, Marco

DOI: https://doi.org/10.1016/j.jvc.2024.04.002

Originally published at:
Ivasovic, Frane; Poletti, Giovanni; Baron Toaldo, Marco (2024). Echocardiographic findings in apparently healthy Czechoslovakian wolfdogs. Journal of Veterinary Cardiology, 53:60-71.
DOI: https://doi.org/10.1016/j.jvc.2024.04.002

Journal of Veterinary Cardiology (2024) **53**, 60–71

ELSEVIER

Journal of Veterinary Cardiology ESVC

www.elsevier.com/locate/jvc

Echocardiographic findings in apparently healthy Czechoslovakian wolfdogs☆

F. Ivasovic [a], G. Poletti [b], M. Baron Toaldo [a,b,*]

[a] *Division of Cardiology, Clinic for Small Animal Internal Medicine, Vetsuisse Faculty University of Zürich, Winterthurerstrasse 260, 8057, Zürich, Switzerland*
[b] *Department of Veterinary Medical Sciences, Alma Mater Studiorum — University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy*

Received 20 January 2023; received in revised form 26 March 2024; accepted 3 April 2024

KEYWORDS
Canine;
Myxomatous mitral valve disease;
Reference intervals;
Subaortic stenosis

Abstract *Introduction:* To echocardiographically evaluate a large number of apparently healthy Czechoslovakian wolfdogs (CWDs) to identify possible subclinical cardiac abnormalities and to generate reference intervals.
Animals: One-hundred and seventeen apparently healthy client-owned CWDs.
Materials and methods: Standard two-dimensional, M-mode, and Doppler echocardiographic measurements were obtained on non-sedated, manually restrained standing dogs. Animals with no relevant echocardiographic abnormalities were used to generate reference intervals. Echocardiographic variables were compared between males and females and between dogs with and without mitral regurgitation (MR).
Results and discussion: Among the 117 CWDs, 103 dogs were used to generate reference intervals. The 14 dogs with abnormalities had more than trivial MR (12 dogs), subaortic stenosis (one dog), and equivocal subaortic stenosis (one dog). The 44 males were heavier than 59 females ($P<0.001$) and had a larger maximum left atrial dimension ($P=0.015$), left ventricular internal dimension at end-diastole ($P<0.001$) and systole ($P<0.001$), and thicker interventricular septum thickness at end-diastole ($P=0.016$). A positive linear correlation was identified between bodyweight and aortic root and left atrial diameters and left ventricular dimensions and between age and aortic root and left atrial diameter and peak late transmitral flow

☆ A unique aspect of the Journal of Veterinary Cardiology is the emphasis of additional web-based materials permitting the detailing of procedures and diagnostics. These materials can be viewed (by those readers with subscription access) by going to http://www.sciencedirect.com/science/journal/17602734. The issue to be viewed is clicked and the available PDF and image downloading is available via the Summary Plus link. The supplementary material for a given article appears at the end of the page. To view the material is to go to http://www.doi.org and enter the doi number unique to this paper which is indicated at the end of the manuscript.

* Corresponding author.
E-mail address: marco.barontoaldo@uzh.ch (M. Baron Toaldo).

https://doi.org/10.1016/j.jvc.2024.04.002

velocity. A negative linear correlation was identified between age and peak early transmitral flow velocity and the ratio between peak early and late transmitral flow velocities. No differences in echocardiographic-derived cardiac dimensions were detected between healthy dogs and dogs with more than trivial MR.
Conclusions: In this population of CWDs, subclinical cardiac abnormalities were uncommon, and they were mainly classified as MR.

Abbreviations

CWD	Czechoslovakian wolfdogs
GSD	German shepherd dog
MR	mitral regurgitation
SCA	subclinical cardiac abnormality

Introduction

Czechoslovakian wolfdogs (CWDs) originate from the Slovakian Republic and are a crossbreed between German shepherd dogs (GSDs) and the Carpathian wolf.[c] This direct biological linkage may allow the transmission of potential health issues from its ancestors to the CWDs. Like in GSDs, dwarfism has been described and genetically characterized in CWDs [1]. There is little knowledge on cardiac diseases in CWDs besides being mentioned as an examined dog breed in epidemiological studies of congenital anomalies [2,3]. Recently, a genetic study on CWDs identified a mutation in the striatin gene, which could be responsible for the development of arrhythmogenic right ventricular cardiomyopathy [4]. Due to the genetic proximity to GSDs, some cardiac diseases typical of this breed could be expected in CWDs; these include congenital diseases like patent ductus arteriosus [2], subaortic stenosis [5,6], mitral valve dysplasia [7], or persistent left cranial vena cava [8], as well as the risk of developing myxomatous mitral valve disease [9]. Similarly, their direct descendants from the common wolf could predispose the CWDs to cardiac diseases, like mitral valve dysplasia reported in red wolves [10] or myxomatous mitral valve disease reported in gray as well as in red wolves [10,11].

Bodyweight-based and breed-specific echocardiographic reference intervals are useful in clinical practice to better and more precisely assess dogs of peculiar or uncommon breeds [12]. Therefore, the objectives of this study were to describe echocardiographic findings in a large population of clinically healthy CWDs and to identify possible subclinical cardiac abnormalities (SCAs).

Animals, materials and methods

Study population

Purebred CWDs were prospectively enrolled in the Italian territory from May 2018 to February 2019. Animals were recruited by advertising the study through a newsletter of the Italian Kennel Club of the breed, by participating in dog shows, and by verbal information between breeders and owners. All examinations were performed in the field with the animals not sedated and gently manually restrained. Due to the typically aggressive temperament of the breed, all dogs were wearing muzzles and were standing during the examinations. Dogs were deemed healthy based on anamnesis and clinical examination, and they were not allowed to be under pharmacological treatment at the time of and before the examination. A board-certified veterinary cardiologist performed cardiac auscultation. The presence of a murmur was not considered an exclusion criterion as abnormal auscultation findings could accompany SCAs. Dogs were excluded if no pedigree was available to review, if they were too aggressive to be examined, or if they were younger than one year of age.

Echocardiography

All transthoracic echocardiographic examinations[d] were performed by the same cardiologist (MBT) on standing dogs and following standard recom-

[c] FCI (Federation Cynologique Internationale) breed standards for the Czechoslovakian wolfdog http://www.fci.be/en/nomenclature/CZECHOSLOVAKIAN-WOLFDOG-332.html (accessed May 03, 2021).

[d] Vinno 6 VET, VINNO Technology Co, Suzhou, China.

mendations [13]. Left ventricular internal dimensions and wall thickness at end-diastole and end-systole were obtained from an M-mode image recorded from a right parasternal short-axis view at the level of the papillary muscles by using a leading-edge-to-leading edge technique. Left ventricular fractional shortening was calculated using the appropriate formula. Aortic root diameter and left atrial diameter were measured using the standard method from a right parasternal short-axis view on the frame just after aortic valve closure [14]. Peak transpulmonic flow velocity was recorded from the right parasternal short axis at basilar level using the pulsed-wave Doppler. A value <190 cm/s was considered normal [15]. Peak transaortic flow velocity was recorded using the continuous-wave Doppler from a left apical five-chamber view, paying attention to keeping the flow as parallel as possible to the cursor. Due to the standing position of the dogs, subcostal views could not be consistently performed, and therefore, they were removed from the echocardiographic protocol. Mitral valve diastolic inflow waves were recorded from a left apical four-chamber view with the pulsed-wave Doppler cursor on the tip of the valve. Peak early and late diastolic transmitral flow velocities were measured, and their ratio was calculated. The presence of a subjective thickening of the mitral valve leaflets and prolapse or any other abnormality were annotated. Color Doppler was used to identify mitral regurgitation (MR) from right parasternal four-chamber, five-chamber, and left apical views. The degree of MR was subjectively graded from the left apical view by being trivial (extension of the color jet by a few millimeters from the valvular plane) [16], mild (representing a subjective estimation of the regurgitation jet area to the left atrial area less than 30%), and moderate (an estimate 30–70% regurgitation jet area to left atrial area) [17]. Subaortic stenosis was excluded for a peak transaortic flow velocity of 200 cm/s or less, considered present for a velocity above 250 cm/s, while a value between 200 and 250 cm/s was deemed equivocal [18]. Electrocardiography was synchronous to the echocardiographic examination. All examinations were stored on an external hard disk in digital imaging and communications in medicine format and measured offline by one operator (MBT) blind to the dogs' identity.[e] Each measurement was performed on three consecutive recorded beats, and the means were calculated.

Statistical analysis

Data distributions were evaluated using Shapiro–Wilk test and visual inspection of QQ plots. The mean and standard deviation of each variable was calculated for normally distributed values, while non-normal variables were presented as median and interquartile range. F-tests were used to assess the homogeneity of variances. The *t*-test or Wilcoxon–Mann–Whitney test was used to compare variables between male and female dogs and between dogs with and without MR. For echocardiographic variables, 95% reference intervals were calculated using a robust method for determination of location and scale; 90% confidence intervals about the lower and upper limits of the calculated reference intervals were determined using a bootstrap method [19]. Outliers were determined by using the cook distances. Only dogs with a structurally normal heart and not more than a trivial MR were included. The presence of a hemodynamic non-relevant anomaly (such as persistent left cranial vena cava or imperforated septal defect) was not considered as an exclusion criterion for the generation of reference values. Spearman or Pearson correlations were used to evaluate the relationship between bodyweight, age, and the different echocardiographic variables. To further assess the impact of bodyweight on specific echocardiographic measurements, a linear regression modeling approach was performed; their relationship was evaluated between log-transformed bodyweight and echocardiographic parameters to minimize dimensional inconsistency.

To investigate the relationships between specific echocardiographic parameters and factors such as bodyweight and sex, we employed a multiple linear regression analysis; in our regression models, we included bodyweight, sex, and the interaction term between bodyweight and sex as predictors. To address the potential issue of collinearity, we computed the variance inflation factor. To provide a practical understanding of the interpretation of certain bodyweight-dependent parameters, these were generated by back-transforming the formula $\log_{10}(y) = \log_{10}(x)\, a + b$. A linear relationship was assumed over the narrow of bodyweights in order to determine the clinical relevance of the sex and bodyweight effects. Dogs with a relevant MR (mild or more)

[e] TomTec Arena, TOMTEC Imaginig Systems GmbH, Unterschleissheim, Germany.

and without other cardiac pathologies were included in the MR group. The level of significance was set for an alpha value of 0.05. This part of the analysis was performed using a dedicated software program.[f,g]

Results

A total of 117 CWDs were included. Of these, 68 were females and 49 were males. One hundred and three dogs (including 15 dogs with trivial MR) had no relevant cardiac abnormalities, and they were used for generating reference intervals (Table 1). This population comprised 59 of 103 females (58 intact and one neutered) and 44 of 103 intact males; their ages ranged from one year to 12.7 years (median: 2.3 years). Males (34.5 kg [26.0—46.0]) were heavier than females (28.0 kg [21.0—40.0]) ($P<0.001$), while no difference was found among ages ($P=0.488$). Some echocardiographic variables differed between male and female CWDs with no relevant cardiac abnormalities (Table 2).

A systolic heart murmur was auscultated in eight of 117 dogs. Murmur intensity varied between three of six (one dog had subaortic stenosis, and one dog had no SCA), two of six (three dogs had mild to moderate MR, two dogs had no SCA), and one of six dogs (one dog had no SCA). Among the four of eight dogs with no clear explanation for the heart murmur, peak transaortic flow velocity was 145 cm/s (130—184) and peak transpulmonic flow velocity was 120 cm/s (90—150). Therefore, 50% of the auscultated heart murmurs in this population were functional murmurs, while the overall prevalence was 3.4%.

Among the 14 dogs with SCAs, one dog had a subaortic stenosis, one dog had an equivocal subaortic stenosis, and 12 dogs had more than trivial MR. One dog was presented with a small imperforated interventricular septal aneurism. In four dogs, a persistent left cranial vena cava was identified. This was diagnosed when a tubular cavitary structure was visible along the lateral and posterior left atrial walls and ending into a dilated coronary sinus [20]. These last five dogs were included in the population to generate reference intervals as these abnormalities were considered irrelevant. In 27 of 117 dogs, an MR was documented. Of these, 15 had a trivial MR and were therefore regarded as having no relevant cardiac abnormalities. The other 12 dogs had a more than trivial MR: eight of 12 dogs had a mild MR, while four of 12 had a moderate MR (Fig. 1).

Mitral valve thickening was noted in 10 dogs with more than trivial MR and in three dogs without MR or only trivial MR (these three dogs were also retained for generating reference intervals). In comparison, mitral valve prolapse was present in six dogs within the MR group and in six dogs without MR or only trivial MR (these six dogs were also retained for generating reference intervals). Therefore, mitral valve abnormalities, including one or more of more than trivial MR, thickening, and prolapse, were detected in 20 of 117 (17%) dogs included in this study. Dogs without MR (88 dogs) and trivial (15 dogs) MR were younger ($P=0.003$) and had a thicker left ventricular free wall diameter in systole ($P=0.047$) than dogs with MR (12 dogs). No differences between dogs with and without MR were detected among other echocardiographic parameters.

Significant correlations with age were observed for left atrial diameter ($P=0.031$, $r=0.212$), aortic root diameter ($P<0.001$, $r=0.320$), peak early ($P<0.001$, $r=-0.337$) and late ($P<0.001$, $r=0.366$) diastolic transmitral flow velocities, as well as their ratio ($P<0.001$, $r=-0.502$). Significant linear associations with bodyweight were present for left atrial diameter, aortic root diameter, interventricular septum thickness in diastole and in systole, left ventricular internal diameter in diastole and in systole, and left ventricular posterior wall thickness in diastole and in systole (Supplement Fig. 1). Regression data with slopes and intercepts are presented in Table 3, while predicted values and 95% prediction intervals of echocardiographic variables for different classes of bodyweight are reported in Table 4.

Multiple linear regression analysis revealed a significant positive relationship with bodyweight for the left ventricular internal diameter in diastole ($\beta=0.288$, $P=0.001$), the aortic root diameter ($\beta=0.263$, $P<0.001$), and the left atrial diameter ($\beta=0.235$, $P=0.004$); the interventricular septum thickness in diastole and the left ventricular internal diameter in systole did not show a significant relationship with bodyweight in the multiple linear regression analysis; the obtained variance inflation factors for the interaction term between sex and bodyweight were 50.379 and 0.019, respectively. None of the investigated parameters showed a significant impact of sex or any interaction between sex and bodyweight.

Discussion

Our study reports the normal values of selected echocardiographic parameters for CWDs.

[f] R v3.5.1, R Development Core Team, Vienna, Austria.
[g] Prism 7, GraphPad Software Inc., San Diego, CA, USA.

Table 1 Echocardiographic variables in 103 Czechoslovakian wolfdogs without relevant cardiac abnormalities.

Variable	Mean ± SD	Min–Max	95% RI	90% CI about the lower limit	90% CI about the upper limit
Age (years)	2.3 (1.6–4.5)[a]	1.0–12.7			
Bodyweight (kg)	31.2 ± 5.3	21.0–46.0			
SAX LA (mm)	33.1 ± 3.8	24.7–42.9	25.4–40.7	24.4–26.3	39.6–41.8
SAX Ao (mm)	24.7 ± 2.2	19.2–30.5	20.2–28.9	19.6–20.7	28.3–29.6
LA:Ao	1.34 ± 0.14	1.07–1.69	1.07–1.62	1.03–1.09	1.58–1.65
IVSd (mm)	10.5 ± 1.6	6.0–13.8	7.4–13.7	6.9–7.8	13.2–14.2
LVIDd (mm)	41.0 ± 3.2	34.0–49.2	34.5–47.3	33.7–35.3	46.4–48.2
LVPWd (mm)	10.1 ± 1.3	7.2–13.2	7.4–12.8	7.1–7.8	12.4–13.1
IVSs (mm)	13.3 ± 1.9	9.0–18.0	9.4–17.1	8.9–9.9	16.5–17.6
LVIDs (mm)	28.9 ± 3.2	20.5–37.0	22.4–35.2	21.6–23.3	34.2–36.2
LVPWs (mm)	13.1 ± 1.8	8.7–18.8	9.3–16.7	8.8–9.8	16.2–17.2
FS (%)	29 ± 6	14–44	17–41	16–19	39–43
Ao V_{max} (cm/s)	149 ± 23	96–200	101–194	95–107	187–202
PA V_{max} (cm/s)	118 ± 19	78–178	79–157	73–84	151–163
MV E Vel (cm/s)	68 (63–76)[a]	44–103	47–90	43–50	87–94
MV A Vel (cm/s)	43 (36–50)[a]	21–76	19–66	16–23	62–70
MV E:A	1.6 (1.3–1.9)[a]	0.8–3.4	0.5–2.6	0.4–0.7	2.4–2.8

Ao V_{max}: peak transaortic flow velocity; CI: confidence interval; FS: fractional shortening; IVSd/IVSs: interventricular septum thickness in diastole/systole; LA:Ao: left atrium to aortic root ratio; LVIDd/LVIDs: left ventricular internal diameter in diastole/systole; LVPWd/LVPWs: left ventricular posterior wall thickness in diastole/systole; Max: maximum; Min: minimum; MV A: peak late diastolic transmitral flow velocity; MV E: peak early diastolic transmitral flow velocity; MV E:A: ratio of peak early and late diastolic transmitral flow velocities; PA V_{max}: peak transpulmonic flow velocity; RIs: reference intervals; SAX Ao: aortic root diameter in short axis; SAX LA: left atrial diameter in short axis; SD: standard deviation.

[a] Not normally distributed variables. Values as reported as median and interquartile range.

Commonly measured values for determining ventricular dimensions fall within the standard ranges for the non-sight hounds [21]. Comparing our values with published references from the closely related GSDs [22], the mean ventricular wall measurements for females and males are similar between both breeds, but the left ventricular diameters are smaller in CWDs than in the GSDs. For example, the mean of the left ventricular diameter in diastole in CWDs reported in our study is 41.0 ± 3.2 mm, while the reported reference value in the GSDs is 49.5 ± 4.7 mm [22]. This difference could be potentially due to an actual variation between the two canids or due to the intrinsic dissimilarity between datasets of different studies.

On the other hand, our obtained values were higher than the data reported on healthy anesthetized captive maned wolves (*Chrysocyon brachyurus*) [23]. This is not surprising if we consider that these canids are smaller than the Carpathian gray wolves used to breed the CWD.[c] Another study reported echocardiographic parameters of 10 Italian gray wolves and one arctic wolf [11]; despite similar mean bodyweight between the CWDs (28.7 kg) and the investigated wolves (25.8 kg), reported values such as left ventricular internal diameter in diastole (48.7 mm) would be suggestive of a bigger heart. However, a direct comparison is still not possible because these wolves were examined under anesthesia with isoflurane, which is known to cause relevant changes in myocardial function [24].

Some echocardiographic parameters, namely aortic root and left atrial diameters and left ventricular dimensions, were correlated with bodyweight. This finding is extensively clarified by human and veterinary literature [25–27] as it is well known that cardiac dimensions follow the increase of body size of the individual [25–27]. We observed an association between age and increasing aortic root and left atrial diameters. This may be due to further growth as the animals age, but it could also be interpreted as a consequence of progressive left atrial dimension in elderly dogs, due to a para-physiological impairment of left ventricular filling and consequent increase in left atrial filling pressure [28]. In humans, for example, left atrial size increases with age independently of body size [29,30]. Interestingly, a previous report in healthy dogs [27] found no correlation between age and left heart size parameters. Similarly, the correlation found between age and peak early and late diastolic

Table 2 Sex-specific echocardiographic variables in 103 Czechoslovakian wolfdogs without relevant cardiac abnormalities.

Variable	Female (N = 59)					Male (N = 44)					
	Mean ± SD	Min–Max	95% RIs	90% CI about the lower limit	90% CI about the upper limit	Mean ± SD	Min–Max	95% RIs	90% CI about the lower limit	90% CI about the upper limit	P value
Age (years)	2.3 (1.4–4.2)[a]	1.0–8.9				2.5 (1.7–4.8)[a]	1.0–12.7				0.488
Bodyweight (kg)	28.7 ± 4.1	21.0–40.0				34.5 ± 5.0	26.0–46.0				<0.001
SAX LA (mm)	32.3 ± 3.6	24.7–39.5	25.0–39.5	23.8–26.3	38.3–40.9	34.1 ± 3.9	27.5–42.9	26.0–42.1	24.3–27.3	40.6–43.9	0.015
SAX Ao (mm)	24.3 ± 2.0	19.2–28.6	20.1–28.3	19.4–20.8	27.6–29.3	25.2 ± 2.3	20.5–30.5	20.3–29.9	19.3–21.2	28.9–31.2	0.031
LA:Ao	1.33 ± 0.14	1.07–1.69	1.05–1.61	1.00–1.09	1.56–1.67	1.36 ± 0.14	1.1–1.64	1.08–1.64	1.02–1.13	1.58–1.69	0.394
IVSd (mm)	10.2 ± 1.5	6.0–13.4	7.2–13.3	6.6–7.7	12.8–13.9	10.9 ± 1.6	7.7–13.8	7.7–14.1	7.1–8.4	13.5–14.8	0.016
LVIDd (mm)	39.9 ± 2.9	34.0–46.2	33.9–45.8	32.9–34.9	44.8–47.0	42.5 ± 2.9	36.2–49.2	36.2–48.4	35.0–37.5	47.0–50.0	<0.001
LVPWd (mm)	9.9 ± 1.2	7.2–12.4	7.5–12.4	7.0–7.9	12.0–12.8	10.4 ± 1.5	8.1–13.2	7.3–13.3	6.7–7.8	12.7–14.0	0.101
IVSs (mm)	13.1 ± 1.9	9.3–18.0	9.1–16.7	8.4–9.7	15.9–17.5	13.6 ± 1.9	9.0–17.9	9.7–17.6	8.8–10.5	16.9–18.5	0.162
LVIDs (mm)	27.9 ± 2.9	20.5–34.8	22.2–33.8	21.1–23.3	32.7–34.9	30.2 ± 3.2	22.8–37.0	23.5–36.6	22.2–24.7	35.1–38.3	<0.001
FS (%)	30 ± 6	18–44	18–41	16–20	39–43	29 ± 6	14–43	16–41	13–18	38–44	0.319
LVPWs (mm)	12.9 ± 1.6	9.5–16.1	9.7–16.3	9.0–10.2	15.7–16.9	13.2 ± 2.1	8.7–18.8	8.8–17.3	7.9–9.7	16.5–18.4	0.449
Ao V_{max} (cm/s)	146 ± 23	96–196	96–191	87–102	181–201	153 ± 23	111–200	104–200	95–112	189–212	0.123
PA V_{max} (cm/s)	118 ± 19	78–158	78–157	71–85	151–164	120 ± 20	84–178	76–158	66–85	148–168	0.578
MV E Vel (cm/s)	72 (63–79)[a]	44–103	46–96	41–50	91–101	67.5 (62–69)[a]	53–86	50–82	46–53	79–86	0.060
MV A Vel (cm/s)	44 (36–52)[a]	26–76	19–70	15–23	65–76	39.5 (36–47)[a]	21–65	18–62	13–22	57–68	0.151
MV E:A	1.6 (1.3–2.0)[a]	0.8–3.4	0.5–2.8	0.2–0.7	2.5–3.0	1.6 (1.4–1.8)[a]	1.0–3.2	0.6–2.5	0.3–0.9	2.3–2.8	0.986

Ao V_{max}: peak transaortic flow velocity; CI, confidence interval; FS%: fractional shortening; IVSd/IVSs: interventricular septum thickness in diastole/systole; LA:Ao: left atrium to aortic root ratio; LVIDd/LVIDs: left ventricular diameter in diastole/systole; LVPWd/LVPWs: left ventricular free wall diameter in diastole/systole; Max: maximum; Min: minimum; MV A: peak late diastolic transmitral flow velocity; MV E: peak early diastolic transmitral flow velocity; MV E:A: ratio of peak early and late diastolic transmitral flow velocities; PA V_{max}: peak transpulmonic flow velocity; RIs: reference intervals; SAX Ao: aortic root diameter in short axis; SAX LA: left atrial diameter in short axis; SD: standard deviation.

[a] Not normally distributed variables. Values as reported as median and interquartile range.

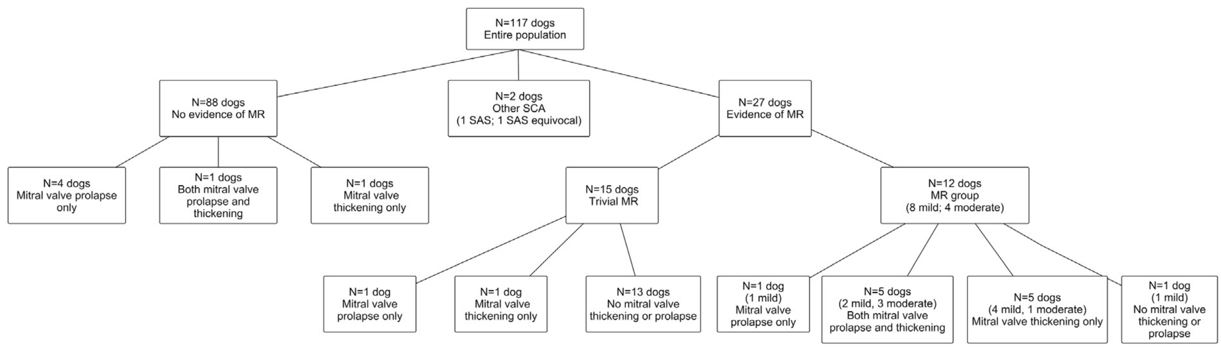

Figure 1 Schematic representation of the echocardiographic diagnosis in a population of apparently healthy Czechoslovakian wolfdogs, focusing on the mitral valve findings. MR: mitral regurgitation; SAS: subaortic stenosis; SCA: subclinical cardiac abnormality.

Table 3 Regression analysis of selected echocardiographic variables with bodyweight from 103 Czechoslovakian wolfdogs without relevant cardiac abnormalities using the equation $\log_{10}(y)=\log_{10}(x)a + b$, where y represents the echocardiographic response variable, x represents the explanatory variable bodyweight, a represents the slope, and b represents the intercept.

Echocardiographic variable	Slope	Intercept	R^2	P value
SAX LA	0.2994	1.0715	0.1797	<0.0001
SAX Ao	0.2860	0.9655	0.2890	<0.0001
IVSd	0.3262	0.5318	0.1175	0.0002
LVIDd	0.2597	1.2253	0.3054	<0.0001
LVPWd	0.3204	0.5242	0.1543	<0.0001
IVSs	0.3355	0.6198	0.1409	<0.0001
LVIDs	0.2083	1.1489	0.0908	0.0012
LVPWs	0.2660	0.7159	0.0908	0.0012

IVSd/IVSs: interventricular septum thickness in diastole/systole; LVIDd/LVIDs: left ventricular diameter in diastole/systole; LVPWd/LVPWs: left ventricular free wall diameter in diastole/systole; SAX Ao: aortic root diameter in short axis; SAX LA: left atrial diameter in short axis.

transmitral flow velocities and their ratio is a described consequence of increased ventricular stiffness in elderly healthy humans [31—34], and the same pattern was noted in dogs in other studies [35—37].

Left ventricular diameter in diastole and systole, as well as the left atrium size and the interventricular septum thickness in diastole, were different between males and females; the bodyweight variation between genders can cause this. Similarly, several echocardiographic parameters analyzed in other breeds [38—40] were also reported to be correlated with bodyweight. However, bodyweight alone cannot always explain echocardiographic sex differences [40]. In people, for example, it is known that left atrial size is not only influenced by body surface area but also by sex [41], which can be potentially explained by the effect of estrogen on the heart [42]. The multiple linear regression analysis showed that the effect of sex and the interaction term between bodyweight and sex had no significant effect on our population, attesting that bodyweight is a significant predictor for certain echocardiographic parameters, while sex might have been confounded by collinearity, as confirmed by the high variance inflation factor.

In our study, four dogs (about 4%) had a soft (<4/6) systolic heart murmur not associated with any SCA. Functional murmurs are described in dogs and cats and are thought to be primarily secondary to decreased blood viscosity or increased cardiac output [43]. The prevalence of functional murmurs in the overall canine young population is reported to be between 15 and 28% [44], which is higher than what was reported in our study. Some canine breeds are more prone to show functional heart murmurs, such as greyhounds [45], whippets [43], and boxers [46]. Prevalence data of functional murmurs in gray wolves [11] are not available, but in a study of 13 maned wolves, only one had a functional murmur [23]. The reason why some CWDs in our study showed functional murmurs could be due to their narrow chest conformation, increased stress during the examination, or potential subclinical anemia [44].

Table 4 Predicted values and 95% prediction intervals for weight-specific echocardiographic linear measurements in 103 Czechoslovakian wolfdogs without relevant cardiac abnormalities.

Bodyweight (kg)	SAX LA (mm)	SAX Ao (mm)	IVSd (mm)	LVIDd (mm)	LVPWd (mm)	IVSs (mm)	LVIDs (mm)	LVPWs (mm)
20	28.91 (23.29 −35.88)	21.76 (18.67 −25.36)	9.04 (6.71 −12.18)	36.58 (32 −41.82)	8.73 (6.78 −11.24)	11.38 (8.62 −15.03)	26.29 (21.16 −32.68)	11.54 (8.74 −15.22)
25	30.9 (25.02 −38.17)	23.19 (19.97 −26.93)	9.72 (7.27 −13.01)	38.77 (34.02 −44.18)	9.38 (7.33 −12)	12.27 (9.35 −16.09)	27.55 (22.28 −34.06)	12.24 (9.34 −16.05)
30	32.64 (26.47 −40.25)	24.43 (21.06 −28.35)	10.32 (7.73 −13.77)	40.65 (35.7 −46.28)	9.94 (7.78 −12.7)	13.04 (9.96 −17.08)	28.61 (23.18 −35.32)	12.85 (9.82 −16.82)
35	34.18 (27.7 −42.17)	25.54 (22 −29.64)	10.85 (8.12 −14.5)	42.31 (37.15 −48.19)	10.45 (8.17 −13.35)	13.74 (10.48 −18)	29.55 (23.92 −36.5)	13.39 (10.22 −17.53)
40	35.57 (28.78 −43.97)	26.53 (22.83 −30.83)	11.33 (8.46 −15.18)	43.8 (38.41 −49.95)	10.9 (8.51 −13.97)	14.36 (10.94 −18.87)	30.38 (24.55 −37.6)	13.87 (10.57 −18.21)
45	36.85 (29.73 −45.68)	27.44 (23.57 −31.95)	11.78 (8.76 −15.83)	45.16 (39.54 −51.58)	11.32 (8.81 −14.55)	14.94 (11.34 −19.7)	31.13 (25.09 −38.63)	14.31 (10.86 −18.86)
50	38.03 (30.58 −47.29)	28.28 (24.23 −33)	12.19 (9.02 −16.46)	46.41 (40.56 −53.12)	11.71 (9.08 −15.11)	15.48 (11.7 −20.49)	31.82 (25.56 −39.62)	14.72 (11.13 −19.47)

IVSd/IVSs: interventricular septum thickness in diastole/systole; LVIDd/LVIDs: left ventricular diameter in diastole/systole; LVPWd/LVPWs: left ventricular free wall diameter in diastole/systole; SAX Ao: aortic root diameter in short axis; SAX LA: left atrial diameter in short axis.

Persistent left cranial vena cava was diagnosed in about 3% of CWDs, using the criteria chosen for this study. This prevalence is higher than that in previous reports in dogs (0.5%) [2] and humans (0.5—2%) [47]. Although the prevalence of this anomaly in GSDs is not available for direct comparison [8], another publication on GSDs with patent ductus arteriosus showed that 10.7% of the dogs included also had a concomitant persistent left cranial vena cava [48], suggesting a possible breed predisposition. The reason why the vascular anomaly has been so frequently observed in CWDs is unclear. One possible explanation is that a genetic mutation led to this malformation. Considering the limited number of CWDs still present in the territory, the diseases have been then transmitted and consolidated in the genetic lines. However, since the definitive diagnosis of persistent left cranial vena cava should be carried out using computed tomography or invasive angiography, it might be that the malformation is overestimated in our study.

An MR was identified in a total of 27 dogs. This was classified as trivial in 15 dogs and mild to moderate in 12 dogs. When assessing myxomatous mitral valve disease prevalence in dogs, body size and breed ancestry play an important role [49]. Since CWDs are directly related to GSDs, which appears to be predisposed to myxomatous mitral valve disease [9,50,51], an increased prevalence could also be expected in CWDs. In our CWD population, excluding cases with trivial mitral insufficiency, the prevalence of MR was 10%. This is higher than the average prevalence of 4.5% [52] and 3.5% [50] reported in previous studies but at the same time lower than that reported in small breeds [53]. Not only the breed but also the age [54] plays a role on the prevalence of mitral valve disease; considering that the dogs in our study were mostly young, an underestimation of the true prevalence could be possible. Myxomatous mitral valve disease is known to be present in wild canids as well, as documented in necroscopic findings in red wolves [10], and in an anesthetized arctic wolf [11]. Interestingly, in this same study [11], a trivial mitral valve insufficiency was identified in five other wolves with no clear changes of the mitral valve apparatus, similarly to 15 CWDs in our study. This might suggest a possible intrinsic and real predisposition to MR in wolves, and not only be due to a side effect of the anesthesia. As mentioned in the most recent American College of Veterinary Internal Medicine consensus guidelines [55], the presence of a myxomatous mitral valve disease should require a regular monitoring in these animals to detect a relevant progression. Nonetheless, in our study, the cardiac dimensions of the dogs with MR did not differ from the other CWDs. This might indicate a potentially less aggressive form of myxomatous mitral valve disease compared to GSDs [9], but it could also be due to the low sample size of CWDs in the MR group or the average young age of our sample. Alternatively, a mild mitral valve insufficiency may be considered physiological rather than pathological in some cases. In humans, a physiological mitral insufficiency can be present as an anatomical variation of normal mitral valve leaflets [56]. Additionally, mild mitral regurgitation is often detected in healthy male endurance athletes, suggesting it may be a normal adaptation to exercise training [57]. A study in Leonbergers identified a high prevalence of a trivial valvular insufficiency [38], suggesting that some canine breeds may have a very mild form of valvular dysplasia that is non-progressive.

In this population of CWDs, we identified one animal with subaortic stenosis and one dog with equivocal subaortic stenosis; therefore, this congenital anomaly appears to be rare. Considering that GSDs are predisposed to subaortic stenosis [6], we cannot exclude that a genetic component is also present in CWDs. Severe subaortic stenosis was not detected in the current study. However, anecdotally, the authors were confronted with young CWDs affected with a severe degree of this disease, which did not reach the adult age. As only adult and apparently healthy dogs were included in the study, an underestimation of the real prevalence of certain congenital anomalies is possible.

A limitation of this study was that ultrasound was performed on standing animals, which could compromise optimal imaging and interfere with measurements. A potential selection bias may have led to the presentation of only dogs that were not suspected of being affected by any cardiac disease or anomaly, leading to a possible underestimation of the prevalence of certain conditions (of congenital or degenerative origin). Nonetheless, more severe pathologies could have been missed due to the inclusion of only adults and clinically healthy CWDs. Due to the study design, it was not possible to assess the variability of the tested variables. Dogs were examined on field conditions, mainly from breed shows, limiting the possibility to scan the same animal repeatedly by the same and different echocardiographers. The cardiologist who performed the examinations was board certified and experienced in echocardiography, and only standard scans and variables were considered, therefore possibly limiting the variability in this study. Nevertheless, reference intervals might be best generated by analyzing independently different populations of animals of the same breed or involving more than one operator in the acquisition and analysis of the exams. Lastly, we decided to include dogs with trivial MR and mitral valve changes not associated with MR in the population used to generate reference intervals. These abnormalities were considered irrelevant since they were not expected to induce any alteration in loading conditions and therefore would not affect chambers dimensions and Doppler variables.

Conclusions

The study reported echocardiographic findings and reference values for CWDs, which are within canine standards. Some SCAs have been observed; among these, MR is the most common cardiac disease observed in the studied population.

Funding

No funding.

Conflict of Interest Statement

The authors do not have any conflict of interest to disclose.

Acknowledgments

We would like to thank the owners of the dogs for allowing their animals to participate in the study.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi https://doi.org/10.1016/j.jvc.2024.04.002.

References

[1] Voorbij AMWY, Leegwater PA, Kooistra HS. Pituitary dwarfism in Saarloos and Czechoslovakian wolfdogs is associated with a mutation in LHX3. J Vet Intern Med 2014;28:1770–4.
[2] Brambilla PG, Polli M, Pradelli D, Papa M, Rizzi R, Bagardi M, Bussadori C. Epidemiological study of congenital heart diseases in dogs: prevalence, popularity, and volatility throughout twenty years of clinical practice. PLoS One 2020;15:e0230160.
[3] Kander M, Pasławska U, Staszczyk M, Cepiel A, Pasławski R, Mazur G, Noszczyk-Nowak A. Retrospective analysis of co-occurrence of congenital aortic stenosis and pulmonary artery stenosis in dogs. Pol J Vet Sci 2015;18:841–5.
[4] Caniglia R, Fabbri E, Hulva P, Bolfíková BČ, Jindřichová M, Stronen AV, Dykyy I, Camatta A, Carnier P, Randi E, Galverni M. Wolf outside, dog inside? The genomic make-up of the Czechoslovakian Wolfdog. BMC Genom 2018;19:533.
[5] Darke PGG. Congenital heart disease in dogs and cats. J Small Anim Pract 1989;30:599–607.
[6] Ontiveros ES, Stern JA. Genetics of canine subvalvular aortic stenosis (SAS). Canine Med Genet 2021;8:4.
[7] Matic SE. Congenital heart disease in the dog. J Small Anim Pract 1988;29:743–59.
[8] Buchanan JW. Persistent left cranial vena cava in dogs: angiocardiography, significance, and coexisting anomalies. Vet Radiol 2005;4:1–8.
[9] Borgarelli M, Zini E, D'Agnolo G, Tarducci A, Santilli RA, Chiavegato D, Tursi M, Prunotto M, Häggström J. Comparison of primary mitral valve disease in German Shepherd dogs and in small breeds. J Vet Cardiol 2004;6:27–34.
[10] Acton AE, Munson L, Waddell WT. Survey of necropsy results in captive red wolves (canis rufus), 1992-1996. J Zoo Wildlife Med 2000;31:2–8.
[11] Guglielmini C, Rocconi F, Brugnola L, Valerio F, Mattei L, Boari A. Echocardiographic and Doppler echocardiographic findings in 11 wolves (*Canis lupus*). Vet Rec 2006;158:125.
[12] Morrison SA, Moise NS, Scarlett J, Mohammed H, Yeager AE. Effect of breed and body weight on echocardiographic values in four breeds of dogs of differing somatotypes. J Vet Intern Med 1992;6:220–4.
[13] Thomas WP, Gaber CE, Jacobs GJ, Kaplan PM, Lombard CW, Moïse NS, Moses BL. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. J Vet Intern Med 1993;7:247–52.
[14] Hansson K, Häggström J, Kvart C, Lord P. Left atrial to aortic root indices using two-dimensional and M-Mode echocardiography in Cavalier King Charles Spaniels with and without left atrial enlargement. Vet Radiol Ultrasoun 2002;43:568–75.
[15] Kirberger RM, Berg PB, Grimbeek RJ. Doppler echocardiography in the normal dog: part II. Vet Radiol Ultrasound 1992;33:380–6.
[16] Boon J. Evaluation of size, function, and hemodynamics. In: Boon JA, editor. Veterinary Echocardiography. 2nd ed. Wiley-Blackwell; 2011. p. 197.
[17] Muzzi RA, de Araújo RB, Muzzi LA, Pena JL, Silva EF. Regurgitant jet area by Doppler color flow mapping: quantitative assessment of mitral regurgitation severity in dogs. J Vet Cardiol 2003;5:33–8.
[18] Ontiveros ES, Stern JA. Genetics of canine subvalvular aortic stenosis (SAS). Canine Med Genet 2021;8:4.
[19] Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Path 2012;41:441–53.
[20] Del Palacio MJF, Bayon A, Agut A. Dilated coronary sinus in a dog with persistent left cranial vena cava. Vet Radiol Ultrasoun 1997;38:376–9.

[21] Esser LC, Borkovec M, Bauer A, Häggström J, Wess G. Left ventricular M-mode prediction intervals in 7,651 dogs: population-wide and selected breed-specific values. J Vet Intern Med 2020;34:2242—52.

[22] Kayar A, Gonul R, Or ME, Uysal A. M-Mode Echocardiographic parameters and indices in the normal German shepherd dog. Vet Radiol Ultrasound 2006;47:482—6.

[23] Estrada AH, Gerlach TJ, Schmidt MK, Siegal-Willott JL, Atkins AL, Gilder JV, Citino SB, Padilla LR. Cardiac evaluation of clinically healthy captive maned wolves (*Chrysocyon brachyurus*). J Zoo Wildlife Med 2009;40:478—86.

[24] Sousa MG, Carareto R, De-Nardi AB, Brito FL, Nunes N, Camacho AA. Effects of isoflurane on echocardiographic parameters in healthy dogs. Vet Anaesth Analg 2008;35: 185—90.

[25] Aurigemma GP, Gottdiener JS, Arnold AM, Chinali M, Hill JC, Kitzman D. Left atrial volume and geometry in healthy aging. Circ Cardiovasc Imag 2009;2:282—9.

[26] Cornell CC, Kittleson MD, Torre PD, Häggström J, Lombard CW, Pedersen HD, Vollmar A, Wey A. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311—21.

[27] Visser LC, Ciccozzi MM, Sintov DJ, Sharpe AN. Echocardiographic quantitation of left heart size and function in 122 healthy dogs: a prospective study proposing reference intervals and assessing repeatability. J Vet Intern Med 2019;33:1909—20.

[28] Blume GG, Mcleod CJ, Barnes ME, Seward JB, Pellikka PA, Bastiansen PM, Tsang, TSM. Left atrial function: physiology, assessment, and clinical implications. Eur J Echocardiogr 2011;12:421—30.

[29] Gardin JM, Henry WL, Savage DD, Ware JH, Burn C, Borer JS. Echocardiographic measurements in normal subjects: evaluation of an adult population without clinically apparent heart disease. J Clin Ultrasound 1979;7:439—47.

[30] Kitzman DW. Normal age-related changes in the heart: relevance to echocardiography in the elderly. Am J Geriatr Cardiol 2000;9:311—20.

[31] Miyatake K, Okamoto M, Kinoshita N, Owa M, Nakasone I, Sakakibara H, Nimura Y. Augmentation of atrial contribution to left ventricular inflow with aging as assessed by intracardiac Doppler flowmetry. Am J Cardiol 1984;53:586—9.

[32] Kitzman DW, Sheikh KH, Beere PA, Philips JL, Higginbotham MB. Age-related alterations of Doppler left ventricular filling indexes in normal subjects are independent of left ventricular mass, heart rate, contractility and loading conditions. J Am Coll Cardiol 1991;18:1243—50.

[33] Gardin JM, Arnold AM, Bild DE, Smith VE, Lima JAC, Klopfenstein HS, Kitzman DW. Left ventricular diastolic filling in the elderly: the cardiovascular health study. Am J Cardiol 1998;82:345—51.

[34] Strait JB, Lakatta EG. Aging-associated cardiovascular changes and their relationship to heart failure. Hear Fail Clin 2012;8:143—64.

[35] Schober KE, Fuentes VL. Effects of age, body weight, and heart rate on transmitral and pulmonary venous flow in clinically normal dogs. Am J Vet Res 2001;62:1447—54.

[36] Garncarz M, Parzeniecka-Jaworska M, Czopowicz M. The influence of age, gender and weight on transthoracic echocardiographic evaluation of transmitral and left ventricular outflow tract diastolic parametersin healthy dogs. Pol J Vet Sci 2019;22:43—9.

[37] Stepien RL, Kellihan HB, Visser LC, Wenholz L, Fuentes VL. Echocardiographic values for normal conditioned and unconditioned North American whippets. J Vet Intern Med 2023;37:844—55.

[38] Stack JP, Fries RC, Kruckman L, Schaeffer DJ. Reference intervals and echocardiographic findings in Leonberger dogs. J Vet Cardiol 2020;29:22—32.

[39] Tsai C-H, Huang C-C, Ho C-C, Claretti M. Echocardiographic parameters and indices in 23 healthy Maltese dogs. J Vet Sci 2021;22:e60.

[40] Crippa L, Ferro E, Melloni E, Brambilla P, Cavalletti E. Echocardiographic parameters and indices in the normal Beagle dog. Lab Anim 1992;26:190—5.

[41] Nikitin NP, Witte KKA, Thackray SDR, Goodge LJ, Clark AL, Cleland JGF. Effect of age and sex on left atrial morphology and function. Eur J Echocardiogr 2003;4:36—42.

[42] Mahmoodzadeh S, Fliegner D, Dworatzek E. Sex and gender differences in pharmacology. Handb Exp Pharmacol 2012; 214:23—195.

[43] Bavegems VC, Duchateau L, Polis IE, Van Ham LM, De Rick AF, Sys SU. Detection of innocent systolic murmurs by auscultation and their relation to hematologic and echocardiographic findings in clinically normal Whippets. J Am Vet Med Assoc 2011;238:468—71.

[44] Szatmári V, Leeuwen MW, Teske E. Innocent cardiac murmur in puppies: prevalence, correlation with hematocrit, and auscultation characteristics. J Vet Intern Med 2015;29: 1524—8.

[45] Fabrizio F, Baumwart R, Iazbik MC, Meurs KM, Couto CG. Left basilar systolic murmur in retired racing Greyhounds. J Vet Intern Med 2006;20:78—82.

[46] Koplitz SL, Meurs KM, Spier AW, Bonagura JD, Fuentes VL, Wright NA. Aortic ejection velocity in healthy Boxers with soft cardiac murmurs and Boxers without cardiac murmurs: 201 cases (1997—2001). J Am Vet Med Assoc 2003;222: 770—4.

[47] Sheikh AS, Mazhar S. Persistent left superior vena cava with absent right superior vena cava: review of the Literature and Clinical Implications. Echocardiography 2014;31: 674—9.

[48] Wesselowski S, Saunders AB, Gordon SG. Anatomy, baseline characteristics, and procedural outcome of patent ductus arteriosus in German Shepherd dogs. J Vet Inter Med 2019;33:471—7.

[49] Parker HG, Kilroy-Glynn P. Myxomatous mitral valve disease in dogs: does size matter? J Vet Cardiol 2012;14: 19—29.

[50] Thrusfield MV, Aitken CGG, Darker PGG. Observations on breed and sex in relation to canine heart valve incompetence. J Small Anim Pract 1985;26:709—17.

[51] Mattin MJ, Boswood A, Church DB, López-Alvarez J, McGreevy PD, O'Neill DG, Thomson PC, Brodbelt DC. Prevalence of and risk factors for degenerative mitral valve disease in dogs attending primary-care veterinary practices in England. J Vet Intern Med 2015;29:847—54.

[52] Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. Adv Vet Sci Comp Med 1977;21:75—106.

[53] Serfass P, Chetboul V, Sampedrano CC, Nicolle A, Benalloul T, Laforge H, Gau C, Hébert C, Pouchelon JL, Tissier R. Retrospective study of 942 small-sized dogs: prevalence of left apical systolic heart murmur and left-sided heart failure, critical effects of breed and sex. J Vet Cardiol 2006;8:11—8.

[54] Whitney JG. Observations on the effect of age on the severity of heart valve lesions in the dog. J Small Anim Pract 1974;15:511—22.

[55] Keene BW, Atkins CE, Bonagura JD, Fox PR, Häggström J, Fuentes VL, Oyama MA, Rush JE, Stepien R, Uechi M. ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. J Vet Intern Med 2019;11:1127—40.
[56] Cebeci B, Kardesoglu E, Celik T, Demiralp E. Echocardiographical characteristics of healthy young subjects with physiological mitral regurgitation. J Int Med Res 2003;32:240—4.
[57] van Buuren F, Gati S, Sharma S, Papadakis M, Adami PE, Niebauer J, et al. Athletes with valvular heart disease and competitive sports: a position statement of the Sport Cardiology Section of the European Association of Preventive Cardiology. Eur J Prev Cardiol 2021:zwab058.

Available online at www.sciencedirect.com
ScienceDirect