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Influence of different fixation protocols on the preservation and dimensions of cardiac tissue

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Abstract

Recent extensive progress in invasive cardiac procedures has triggered a wave of dozens of heart morphometric anatomical studies that are carried out largely using autopsied samples fixed in formaldehyde solution prior to observations and measurements. In reality, very little is known about changes in heart tissue dimensions during fixation. The aim of this study was therefore to investigate how fixation affects the dimensions of cardiac tissue, and if different types and concentrations of reagents affect this phenomenon. A total of 40 pig heart samples were investigated, and seven different measuring sites were permanently marked in every heart prior to fixation. Four study groups ($n = 10$ each) were assembled that differed only in concentration and the type of fixative: (i) 2% formaldehyde solution; (ii) 4% formaldehyde solution (formalin); (iii) 10% formaldehyde solution; (iv) alcoholic formalin. The samples were measured before and after fixation at the following time points: 24 h, 72 h and 168 h. It was found that different fixatives significantly affected different parameters. Almost all of the heart dimensions that were measured stabilized after 24 h; later changes were statistically insignificant in the point-to-point comparison. Change in the length of the interatrial septum surface was not altered significantly in any of the fixatives after 24 h of preservation. It was found that 10% formaldehyde increased the thickness of muscular tissue only after 24 h; this thickening was reduced after 72 h and was insignificant at 168 h. Other heart parameters in this group do not present significant changes over the entire fixation time duration. In conclusion, the 10% formaldehyde phosphate-buffered solution appeared to be the best fixative among the fixatives that were studied for cardiac morphometric purposes; this solution caused the smallest changes in tissue dimensions. Measurements should be obtained at least after 1 week of preservation when most parameters exhibit the smallest changes compared with the non-preserved samples.

Key words: formaldehyde; formalin; heart anatomy; morphometry.

Introduction

Formaldehyde fixation is an inexpensive, commonly available method of tissue preservation that is widely used in both clinical work and scientific studies (Thavarajah et al. 2012). Recent extensive progress in invasive cardiac diagnostics and treatment procedures has triggered a wave of dozens of heart architecture morphometric anatomical studies. The aim of these studies is to better understand the cardiac clinical anatomy, and increase the safety and effectiveness of medical interventions (Sánchez-Quintana et al. 2002; Ho & McCarthy, 2010; Noheria et al. 2013; Hołda et al. 2015; Klimek-Piotrowska et al. 2015; Lama et al. 2015).

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Cardiac morphometric studies have been carried out largely using autopsied samples fixed in formaldehyde solution prior to observations and measurements. The main limitation, noted by reviewers and readers of these studies, is that fixation in aldehydes significantly affects the shape and size of the sample; the results of these samples accordingly cannot be used in clinical practice. In reality, very little is known about changes in heart tissue dimensions during fixation (Gerdes et al. 1982). The current goal was therefore to investigate how fixation affects the dimensions of cardiac tissue, and if different types and concentrations of reagents affect this phenomenon.

Materials and methods

This study was designed and conducted at the Department of Anatomy, Jagiellonian University Medical College, Cracow, Poland. A total of 40 pig hearts were dissected up to 1 h after commercial slaughter of the animals (*Sus scrofa f. domestica*) and washed three times in saline solution. No animal was killed deliberately for this study, and all samples were originally intended for use in the food

industry. The hearts were obtained intact. They were cut in the same manner by the authors. The cut was performed on the right and left side of the interventricular septum. The right atrium was opened in a routine way using intercaval incision extending from the orifice of the superior vena cava to the orifice of the inferior vena cava.

The following points were permanently marked in every heart using pins and sutures up to 2 h after the collection of heart samples (Fig. 1): the thickness of the left ventricle, the thickness of the right ventricle, the diameter of the papillary muscle at its base, the length of the chordae tendineae, the diameter of the anterior interventricular artery, the length between two pins on the interatrial septum surface, and the angle between three pins on the epicardial surface of the left ventricle (to investigate the potential rotation of tissue). Following the marking of the study points, the samples were weighed and measured. After measurements of the non-preserved samples were taken, they were immediately immersed in one of the fixatives.

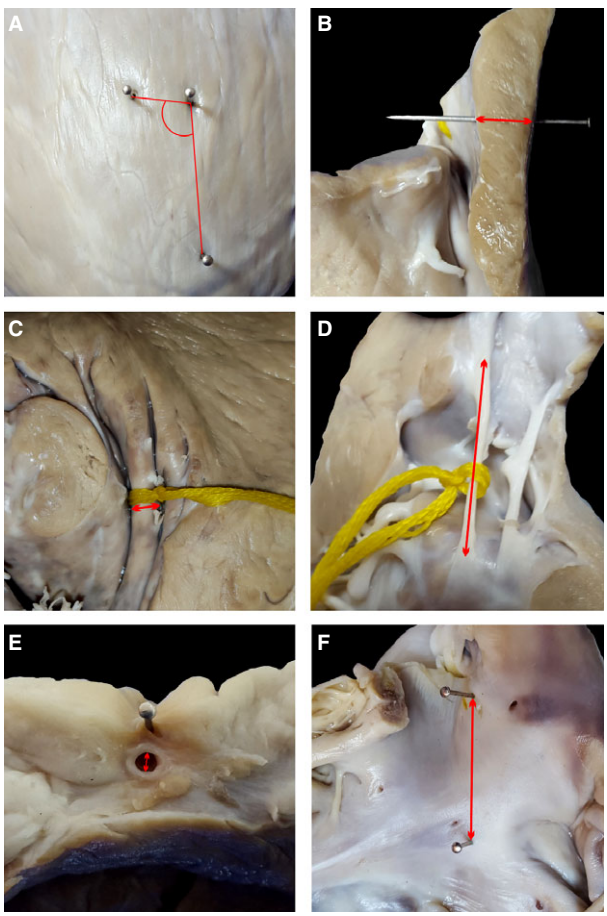

Fig. 1 Photographs of cadaveric heart specimens 24 h after fixation. The following points were permanently marked in every heart using pins and sutures up to 2 h after the collection of heart samples: (A) angle marked on the epicardial surface of the left ventricle; (B) thickness of the right ventricle (the thickness of the left ventricle was marked in the same manner); (C) diameter of the papillary muscle; (D) length of the chordae tendineae; (E) diameter of the anterior interventricular artery; (F) length marked on the interatrial septum surface. The samples were measured before and after fixation at the following time points: 24, 72 and 168 h.

The terms formalin and formaldehyde are often used interchangeably, although it is incorrect to use the two words this way. Formalin is the most commonly used fixative, and it refers to a 37% formaldehyde solution. Therefore, a protocol calling for 10% formalin is roughly equivalent to a 4% formaldehyde solution because 10% buffered formalin is an example of historical jargon describing a 10% solution made from a stock bottle of 37% formaldehyde. In this study, only the term formaldehyde is used to avoid any misunderstanding.

The hearts were randomly assigned to one of four study groups ($n = 10$ each) that differed only in terms of their concentration and fixative type: (i) 2% formaldehyde phosphate-buffered solution (FPBS); (ii) 4% FPBS; (iii) 10% FPBS; (iv) alcoholic formalin solution (Alc; 85.5% absolute ethanol, 3.8% formaldehyde, 0.05% calcium acetate in distilled water). The minimum fixative to tissue ratio was 40 : 1. The maximum thickness of all samples did not exceed 4 cm. Between consecutive measurements, all of the samples were stored at room temperature (21 °C) in sealed containers.

The heart samples were weighed, and then measured again after 24, 72 and 168 h of fixation in the same marked points. For this purpose, the hearts were removed from the fixative solution and dried. All of the linear measurements were collected using 0.03-mm-precision electronic calipers (YATO YT–7201). Angle measurements were obtained using a 1-degree precision half-circle protractor. The measurements of every marked structure at all time points were obtained by two independent researchers in order to minimize human error. In case of a difference exceeding 5% for the same time point, the measurements were repeated.

Statistical analysis was performed using StatSoft STATISTICA 12.0 software for Windows. A *P*-value of less than 0.05 was deemed to be statistically significant. The distributions of the datasets were checked for normality using Shapiro–Wilk tests. Quantile–quantile plots and histograms were evaluated as well if eventually outlier/extreme cases occurred. The data are presented as medians, quantiles (Q1, Q3) and percentages to facilitate illustration of the presented change parameters. Graphs of each variable for all four of the analyzed groups are presented as means with 95% confidence intervals (95% CI).

Friedman's non-parametric test was used to assess whether a certain parameter changed statistically significantly over time in a certain environment. If the aforementioned test result was statistically significant, a *post hoc* analysis was performed in order to check the differences in parameter value between individual time points. The differences (D) in specimen change were compared between 168 h and baseline measurements prior to fixation (D = baseline measurement – 168 h measurement) for each solution in a particular parameter using ANOVA or the Kruskal–Wallis test with *post hoc* analysis.

Results

It was found that different fixatives affected various parameters significantly. Table 1 lists the median values of the measured heart parameters prior to fixation and at consecutive time points using the results of the Friedman test. Table 2 presents relative (percentage) changes in heart weight and the dimensions of the measured structures during the fixation process. Figure 2 shows means with 95% CIs of percentage changes of the measured morphometric parameters of the heart samples preserved in different fixatives at consecutive time points. No

Table 1 Median values of measured heart parameters before fixation and at consecutive time points for samples preserved in different fixatives.

Parameter	Study group	Before fixation Me (Q1; Q3)	24 h Me (Q1; Q3)	72 h Me (Q1; Q3)	168 h Me (Q1; Q3)	*P**
Heart weight (g)	2% FPBS (*n* = 10)	223.5 (209.0; 247.0)	237.5† (220.0; 260.0)	235.5‡ (219.0; 259.0)	233.0§ (217.0; 257.0)	0.000
	4% FPBS (*n* = 10)	225.0 (206.0; 239.0)	231.5† (214.0; 248.0)	229.0‡ (214.0; 247.0)	228.0 (212.0; 245.0)	0.000
	10% FPBS (*n* = 10)	230.0 (210.0; 237.0)	227.0 (212.0; 237.0)	227.0 (210.0; 237.0)	227.0 (209.0; 234.0)	NS
	Alc (*n* = 10)	229.0 (221.0; 263.0)	211.0 (193.0; 241.0)	202.0‡ (187.0; 228.0)	197.0§ (182.0; 221.0)	0.000
Left ventricle thickness (mm)	2% FPBS (*n* = 10)	17.2 (13.5; 20.3)	19.6† (17.6; 23.3)	20.2‡ (15.3; 23.2)	19.1§ (14.8; 22.5)	0.000
	4% FPBS (*n* = 10)	18.0 (15.1; 20.3)	19.3† (18.8; 20.1)	19.0 (16.5; 20.6)	19.1 (16.6; 19.4)	0.04
	10% FPBS (*n* = 10)	18.5 (16.5; 20.1)	20.5† (17.8; 21.3)	20.0 (17.7; 21.1)	19.7 (17.5; 20.4)	0.004
	Alc (*n* = 10)	16.2 (15.1; 17.1)	15.3 (12.7; 18.1)	15.3 (13.8; 18.9)	15.1 (12.7; 18.3)	NS
Right ventricle thickness (mm)	2% FPBS (*n* = 10)	6.1 (4.4; 6.8)	8.0† (7.2; 10.5)	8.3‡ (7.4; 9.1)	7.2 (7.1; 9.2)	0.000
	4% FPBS (*n* = 10)	5.7 (4.4; 6.5)	6.9† (5.8; 7.7)	6.6‡ (5.7; 8.0)	6.4 (5.3; 7.1)	0.000
	10% FPBS (*n* = 10)	5.8 (5.5; 7.3)	6.8† (6.5; 8.0)	6.3 (5.5; 7.5)	6.2 (5.5; 7.4)	0.017
	Alc (*n* = 10)	9.4 (7.1; 9.9)	7.7† (7.1; 8.4)	7.3‡ (7.1; 8.1)	7.5§ (6.7; 8.6)	0.013
Papillary muscle diameter (mm)	2% FPBS (*n* = 10)	5.6 (3.7; 5.8)	6.8† (5.9; 7.1)	6.6‡ (5.9; 7.4)	6.5 (5.2; 7.2)	0.000
	4% FPBS (*n* = 10)	5.3 (4.7; 6.9)	6.1† (4.7; 7.9)	5.5‡ (4.7; 7.5)	5.9§ (5.1; 7.3)	0.04
	10% FPBS (*n* = 10)	6.4 (5.6; 8.6)	7.1† (6.3; 9.6)	6.6 (5.8; 9.8)	6.3 (5.1; 9.5)	0.01
	Alc (*n* = 10)	4.6 (3.4; 5.1)	4.8 (4.1; 5.5)	4.4 (3.9; 5.4)	5.2 (4.0; 6.2)	NS
Chordae tendineae length (mm)	2% FPBS (*n* = 10)	18.9 (15.8; 20.7)	15.4† (12.1; 17.9)	16.7‡ (12.3; 19.4)	16.6§ (12.2; 22.7)	0.003
	4% FPBS (*n* = 10)	18.3 (12.5; 22.4)	12.5† (11.1; 16.2)	13.3‡ (10.3; 17.6)	11.8§ (10.5; 14.3)	0.000
	10% FPBS (*n* = 10)	11.7 (6.7; 13.3)	10.1 (7.4; 13.2)	9.8 (7.0; 11.8)	8.8 (7.7; 13.8)	NS
	Alc (*n* = 10)	14.5 (13.3; 16.2)	10.8† (8.3; 12.5)	10.8‡ (8.2; 11.8)	10.1§ (8.7; 14.7)	0.002
Anterior interventricular artery diameter (mm)	2% FPBS (*n* = 10)	2.7 (2.5; 3.0)	2.8 (2.6; 3.2)	3.2 (2.7; 3.3)	2.8 (2.5; 3.4)	NS
	4% FPBS (*n* = 10)	3.2 (2.8; 3.5)	3.0 (2.7; 3.6)	3.3 (3.1; 3.6)	3.1 (2.7; 3.3)	NS
	10% FPBS (*n* = 10)	3.3 (3.1; 3.5)	3.2 (2.9; 3.4)	3.6 (3.4; 3.8)	3.7 (2.8; 4.0)	NS
	Alc (*n* = 10)	3.1 (2.7; 3.5)	3.1 (2.9; 3.3)	2.8 (2.7; 3.2)	3.0 (2.2; 3.5)	NS
Length on interatrial septum surface (mm)	2% FPBS (*n* = 10)	23.4 (20.0; 24.8)	24.9 (21.7; 25.9)	24.0 (21.8; 25.8)	24.0 (21.5; 26.1)	NS
	4% FPBS (*n* = 10)	24.0 (20.4; 27.1)	24.6 (20.7; 27.7)	23.4‡ (19.6; 26.5)	22.0§ (20.5; 25.6)	0.032
	10% FPBS (*n* = 10)	26.0 (25.3; 26.5)	26.1 (24.2; 27.8)	25.2 (24.0; 26.8)	25.5 (25.0; 26.5)	NS
	Alc (*n* = 10)	19.9 (18.3; 21.1)	20.1 (19.7; 20.6)	20.3 (19.4; 20.7)	20.1 (19.4; 20.6)	NS
Angle (°)	2% FPBS (*n* = 10)	90.0	90.0 (87.0; 91.0)	92.0 (88.0; 96.0)	92.0 (86.0; 97.0)	NS
	4% FPBS (*n* = 10)	90.0	97.5† (90.0; 104.0)	99.0‡ (93.0; 105.0)	99.0§ (93.0; 106.0)	0.007
	10% FPBS (*n* = 10)	90.0	89.0 (84.0; 97.0)	93.0 (85.0; 97.0)	89.0 (84.0; 94.0)	NS
	Alc (*n* = 10)	90.0	91.0 (91.0; 95.0)	93.0 (88.0; 101.0)	91.0 (88.0; 97.0)	NS

Alc, alcoholic formalin solution; FPBS, formaldehyde phosphate-buffered solution; Me, median; *n*, number of samples; Q1 and Q3, lower and upper quartiles.

*Friedman test.

†Repeated-measures analysis of variance evaluating the 24-h time point score compared with the baseline score, $P < 0.05$.

‡Repeated-measures analysis of variance evaluating the 72-h time point score compared with the baseline score, $P < 0.05$.

§Repeated-measures analysis of variance evaluating the 168-h time point score compared with the baseline score, $P < 0.05$.

difference in heart weight prior to fixation was observed across groups ($P = 0.72$).

After 24 h of preservation in 2% formaldehyde solution, an increase in specimen weight [5.0% (4.3; 6.1), $P = 0.000$] and contraction of the chordae tendineae [−12.6% (−17.3; −10.0), $P = 0.003$] were noted. An increased thickness of the muscular tissue (the thicker the muscular tissue is, the smaller the change in dimension that could be observed) was observed [left ventricle: 13.7% (6.4; 22.1), $P = 0.000$; right ventricle: 38.7% (31.5; 73.0), $P = 0.000$; papillary muscle: 33.2% (9.8; 68.6), $P = 0.000$]. In this group, the most pronounced changes in muscular tissue thickness exceeded 50%.

It was found that 4% formaldehyde fixation after 24 h significantly affected the heart weight [3.7% (3.1; 4.1), $P = 0.000$], caused chordae tendineae contraction [−15.9% (−30.2; −9.8), $P = 0.000$], increased the thickness of the muscular tissue [left ventricle: 10.0% (3.2; 20.1), $P = 0.04$; right ventricle: 23.9% (19.1; 33.3), $P = 0.000$; papillary muscle: 16.8% (−1.7; 30.9), $P = 0.04$] and changed the dimension of the angle marked on the heart [8.3% (4.4; 15.6), $P = 0.007$].

It was also noted that the 10% formaldehyde solution increased the thickness of muscular tissue only after 24 h [left ventricle: 9.9% (3.2; 13.7), $P = 0.004$; right ventricle: 11.3% (8.3; 17.6), $P = 0.017$; papillary muscle: 11.6% (10.9; 20.1), $P = 0.01$]. This thickening was reduced after 72 h, and it was insignificant after 168 h. The other heart parameters did not exhibit any statistically significant changes during the course of the fixation time.

Table 2 Relative (percentage) changes in the measured heart parameters at consecutive time points for samples preserved in different fixatives.

Parameter	Study group		24 h Me (Q1; Q3)	72 h Me (Q1; Q3)	168 h Me (Q1; Q3)
Heart weight	2% FPBS (n = 10)	A	5.0% (4.3; 6.1)	4.2% (3.9; 5.3)	3.4% (2.6; 4.3)
		B		−0.6% (−0.9; 0.0)	−0.9% (−0.9; −0.8)
	4% FPBS (n = 10)	A	3.7% (3.1; 4.1)	3.1% (1.8; 3.7)	2.3% (1.7; 2.7)
		B		−0.7% (−1.3; −0.4)	−0.8% (−0.9; −0.4)
	10% FPBS (n = 10)	A	0.0% (−0.4; 1.0)	0.0% (−0.4; 0.0)	−0.5% (−1.3; −0.4)
		B		0.0% (−0.5; 0.0)	−0.5% (−0.5; 0.0)
	Alc (n = 10)	A	−9.4% (−10.9; −8.0)	−13.3% (−15.4; −12.5)	−16.0% (−17.0; −14.3)
		B		−4.3% (−5.4; −3.2)	−2.7% (−3.3; −1.7)
Left ventricle thickness	2% FPBS (n = 10)	A	13.7% (6.4; 22.1)	11.2% (9.2; 14.3)	10.7% (7.9; 13.9)
		B		3.1% (−10.5; 7.2)	−1.3% (−3.0; 1.3)
	4% FPBS (n = 10)	A	10.0% (3.2; 20.1)	3.7% (1.5; 8.2)	3.7% (−1.6; 8.8)
		B		−3.7% (−6.1; 1.5)	−0.5% (−3.1; 2.2)
	10% FPBS (n = 10)	A	9.9% (3.2; 13.7)	8.1% (2.4; 10.9)	6.1% (−4.9; 10.4)
		B		−4.9% (−8.7; 0.6)	−1.1% (−4.4; 0.0)
	Alc (n = 10)	A	−3.2% (−18.8; 2.5)	−3.4% (−19.6; 2.9)	−2.9% (−20.2; −1.4)
		B		0.4% (−0.3; 4.7)	−3.1% (−5.0; −1.0)
Right ventricle thickness	2% FPBS (n = 10)	A	38.7% (31.5; 73.0)	33.3% (27.9; 46.6)	21.7% (16.4; 5.3)
		B		−3.5% (−15.2; 5.7)	−3.8% (−8.9; −1.1)
	4% FPBS (n = 10)	A	23.9% (19.1; 33.3)	26.2% (17.7; 34.2)	17.4% (7.0; 24.4)
		B		0.1% (−5.9; 5.4)	−6.9% (−15.1; −3.8)
	10% FPBS (n = 10)	A	11.3% (8.3; 17.6)	2.2% (−3.2; 7.7)	−1.9% (−5.1; 5.8)
		B		−7.6% (−18.9; −3.9)	0.0% (−3.9; 3.6)
	Alc (n = 10)	A	−12.6% (−22.2; −3.2)	−14.1% (−19.1; 4.0)	−16.0% (−16.5; −13.1)
		B		−5.3% (−10.9; −1.6)	−0.7% (−13.0; 3.9)
Papillary muscle diameter	2% FPBS (n = 10)	A	33.2% (9.8; 68.6)	36.0% (13.3; 67.2)	23.7% (17.9; 44.8)
		B		−0.5% (−2.9; 4.6)	−9.0% (−14.8; 4.1)
	4% FPBS (n = 10)	A	16.8% (−1.7; 30.9)	7.0% (−6.2; 35.4)	18.7% (−1.7; 38.2)
		B		−3.4% (−8.8; 0.0)	0.2% (−3.9; 6.2)
	10% FPBS (n = 10)	A	11.6% (10.9; 20.1)	5.9% (2.7; 18.7)	3.2% (−7.6; 8.7)
		B		−3.4% (−13.4; −2.2)	−2.5% (−9.4; 0.0)
	Alc (n = 10)	A	7.8% (−3.0; 9.8)	−4.7% (−21.9; 4.9)	−4.1% (−19.3; 21.2)
		B		−4.6% (−12.9; −3.3)	10.0% (0.4; 15.5)
Chordae tendineae length	2% FPBS (n = 10)	A	−12.6% (−17.3; −10.0)	−11.7% (−15.5; −9.7)	−12.2% (−16.0; −3.5)
		B		1.9% (−2.2; 5.7)	−0.1% (−5.8; 9.7)
	4% FPBS (n = 10)	A	−15.9% (−30.2; −9.8)	−19.5% (−32.4; −12.8)	−30.0% (−42.1; −20.2)
		B		−0.6% (−7.6; 8.9)	−9.4% (−21.6; −0.9)
	10% FPBS (n = 10)	A	2.6% (−17.8; 15.4)	4.1% (−16.7; 15.0)	−11.6% (−24.4; 4.9)
		B		−5.9% (−18.0; 0.0)	0.9% (−9.4; 16.1)
	Alc (n = 10)	A	−18.8% (−40.8; −13.8)	−18.6% (−45.1; −14.9)	−23.9% (−41.9; −12.4)
		B		−1.2% (−5.6; 0.9)	1.9 (−1.9; 5.8)
Anterior interventricular artery diameter	2% FPBS (n = 10)	A	3.8% (−16.0; 16.7)	13.3% (7.4; 22.7)	4.8% (−11.1; 17.2)
		B		15.5% (0.0; 23.1)	−3.4% (−7.4; 0.0)
	4% FPBS (n = 10)	A	−6.6% (−11.4; 9.1)	10.9% (−6.5; 27.3)	−4.9% (−12.9; 26.9)
		B		17.6% (0.0; 22.2)	−5.0% (−9.7; 0.0)
	10% FPBS (n = 10)	A	−5.3% (−7.1; −1.7)	16.8% (1.4; 18.6)	−12.0% (−20.9; 17.5)
		B		15.8% (6.2; 26.8)	−5.9% (−22.0; 3.1)
	Alc (n = 10)	A	−7.1% (−15.8; 22.7)	−1.2% (−15.8; 1.1)	−10.7% (−18.5; 0.0)
		B		−11.1% (−14.5; 0.0)	−6.2% (−17.0; 18.8)
Length on interatrial septum surface	2% FPBS (n = 10)	A	2.0% (−2.2; 8.5)	0.4% (−3.4; 4.0)	−2.0% (−3.3; 5.7)
		B		−0.2% (−0.8; 0.8)	−0.8% (−1.8; 0.9)
	4% FPBS (n = 10)	A	2.2% (−2.0; 4.1)	−3.2% (−6.8; 1.6)	−0.3% (−10.8; 1.8)
		B		−2.4% (−4.1; −1.1)	0.7% (−1.0; 2.3)
	10% FPBS (n = 10)	A	3.0% (−4.3; 6.7)	−0.3% (−6.4; 1.9)	−1.1% (−3.9; 2.2)
		B		−2.1% (−5.6; −0.8)	−0.5% (−1.2; 4.5)
	Alc (n = 10)	A	3.5% (−2.4; 7.7)	1.6% (−2.9; 6.1)	2.4% (−2.4; 6.0)
		B		−1.0% (−1.4; 0.8)	0.0% (−0.5; 0.8)

Table 2. (continued)

Parameter	Study group		24 h Me (Q1; Q3)	72 h Me (Q1; Q3)	168 h Me (Q1; Q3)
Angle	2% FPBS (n = 10)	A	2.8% (1.1; 6.2)	4.4% (2.2; 6.7)	5.0% (4.4; 7.8)
		B		2.8% (1.1; 6.2)	1.6% (1.0: 4.5)
	4% FPBS (n = 10)	A	8.3% (4.4; 15.6)	10.0% (3.3; 16.7)	10.0% (3.3; 17.8)
		B		1.6% (1.0; 3.3)	2.5% (1.0; 4.4)
	10% FPBS (n = 10)	A	7.8% (6.7; 10.0)	6.7% (5.6; 10.0)	6.7% (4.4; 7.8)
		B		2.4% (1.0; 3.6)	1.3% (1.1; 2.0)
	Alc (n = 10)	A	3.3% (1.1; 6.7)	7.8% (3.3; 13.3)	6.7% (2.2; 10.0)
		B		4.3% (2.2; 5.7)	2.2% (1.0; 4.6)

The data are presented and compared both with values before the preservation process (A) and between specific time intervals (B).A: relative (percentage) changes of parameters in particular time intervals, always compared with results before fixation (baseline); baseline–24 h, baseline–72 h and baseline–168 h; B: relative (percentage) changes of parameters in time intervals in the point-to-point comparison; baseline-24 h, 24–72 h and 72–168 h.
Alc, alcoholic formalin solution; FPBS, formaldehyde phosphate-buffered solution; Me, median; n, number of samples; Q1 and Q3, lower and upper quartiles.

Alcoholic formalin solution caused a reduction in the heart weight [−9.4% (−10.9; −8.0), $P = 0.000$] and right ventricle thickness [−12.6% (−22.2; −3.2), $P = 0.013$], contraction of the chordae tendineae [−18.8% (−40.8; −13.8), $P = 0.002$; the largest among all four of the study groups]. Bidirectional changes in the papillary muscle diameter were also observed that were statistically insignificant.

The anterior intraventricular artery diameter was not affected significantly in any study group. The change in the length of the interatrial septum surface was not altered significantly in any of the fixatives after 24 h of preservation. All of the measured heart dimensions were stabilized after 24 h, and later changes were statistically insignificant in the point-to-point comparison. The only exception was the length of the interatrial septum surface in 4% formaldehyde solution (insignificant change after 24 h and a significant reduction after 72 h that was sustained after 168 h, $P = 0.032$). Interestingly, the repeated-measures analysis of variance evaluating the 24, 72 and 168 h time points score to the baseline score always revealed that some initially significant changes (measured after 24 h) were reduced to statistically insignificant changes after 72 h or even 168 h of fixation.

Differences in changes in the measured parameters between 168 h and the baseline measurements were also investigated before fixation for each solution. No differences in heart weight change were observed only between the 2% and 4% FPBS ($P = 0.39$) solutions; in other solutions, the heart weight differed significantly ($P < 0.01$), with the smallest weight change in the 10% FPBS. The change in the left ventricle thickness, the papillary muscle diameter, the anterior intraventricular artery diameter and the length of the interatrial septum did not differ significantly between the groups. A statistically significant change in the right ventricle thickness was observed between the Alc and all FPBS groups ($P = 0.000$), and between the 2% and 10% FPBS ($P = 0.000$) groups, with the smallest change in the 10% FPBS. The change in chordae tendineae length was significantly larger in comparison between 2% and 4% FPBS ($P = 0.02$), as well as 4% and 10% FPBS ($P = 0.000$), with the smallest change in length in the 10% FPBS. The angle differed significantly only between 4% and 10% FPBS ($P = 0.02$), with the smallest change of parameter for the 10% FPBS.

Discussion

It is commonly known that formaldehyde fixation causes morphometric changes in preserved tissue (Fox et al. 1985). A small number of studies have investigated this problem and provided very little information about the exact nature of these changes; most of these studies were related to tumors (Jonmarker et al. 2006; Chen et al. 2012; Vent et al. 2014; Tran et al. 2015). The practical aspect of this work is the presentation, for the first time, of complex information about changes in cardiac tissue during its preservation. This study eliminates the majority of related limitations present in morphometric examinations of the heart. The animal tissue used in this study did not differ in terms of any of the anatomical aspects that were studied compared with human tissue; therefore; the results of this study can be translated into research on human hearts in their entirety (Crick et al. 1998).

Among all studied fixatives, 10% FPBS seems to be the best fixative for cardiac morphometric studies. This solution did not cause any statistically significant changes in all of the tissue parameters that were investigated after at least 72 h of fixation. For measurements taken from atrial tissue,

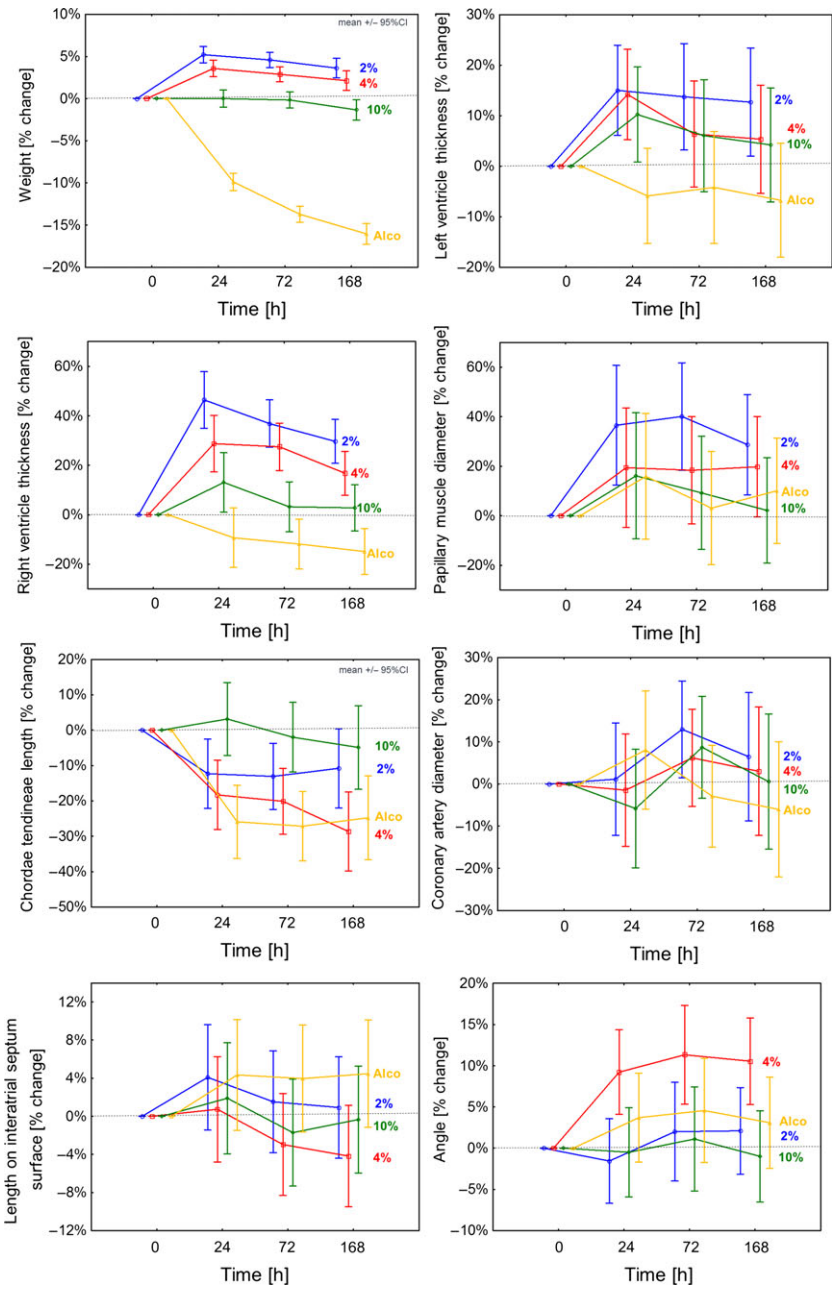

Fig. 2 Graphs of means with 95% confidence intervals (CIs) of the percentage changes of the measured morphometric parameters of the heart samples preserved in different fixatives at consecutive time points. Two percent formaldehyde phosphate-buffered solution (FPBS; $n = 10$); 4% FPBS ($n = 10$); 10% FPBS ($n = 10$), alcoholic formalin solution (Alc; $n = 10$).

all of the studied fixatives are good choices, and no corrections are necessary. For other fixatives and structures, the correction factors derived from the present study (the median values of the percentage changes presented in Table 2) should be taken into consideration to obtain more precise results.

Time is an important factor in fixation. Surprisingly, irrespective of the fixative type, consecutive measurements revealed smaller changes in tissue diameters. Studies conducted after a minimum of a week of preservation reflected the size of the cardiac tissue prior to fixation more accurately. As expected, this study demonstrated thickening of the cardiac muscle mass in PBS-based fixatives (Cutts, 1988), but replacing the phosphate buffer with a high-percentage ethanol led to a significant reduction in the tissue size. Other differences between alcoholic and PBS fluids were related to heart weight (significantly increased in low FPBSs and decreased in alcoholic formalin).

Conclusions

All of the fixatives that were investigated affected various morphometric heart parameters significantly. The 10% FPBS seems to be the best fixative among the fixatives that were studied for cardiac morphometric purposes; it caused the smallest changes in tissue dimensions. Measurements

should be taken at least after 1 week of preservation in whichever fixative is used; many parameters exhibited the smallest changes at this time compared with non-preserved samples.

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Author contributions

M.K.H. design, acquisition of data, data analysis, drafting of the manuscript, critical revision of the manuscript, approval of the article. W.K.P. design, acquisition of data, data analysis, drafting of the manuscript, critical revision of the manuscript, approval of the article. M.K. data analysis, drafting of the manuscript, critical revision of the manuscript, approval of the article. K.P. acquisition of data, drafting of the manuscript, critical revision of the manuscript, approval of the article. J.H. acquisition of data, data analysis, drafting of the manuscript, critical revision of the manuscript, approval of the article.

Conflicts of interest

None.

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