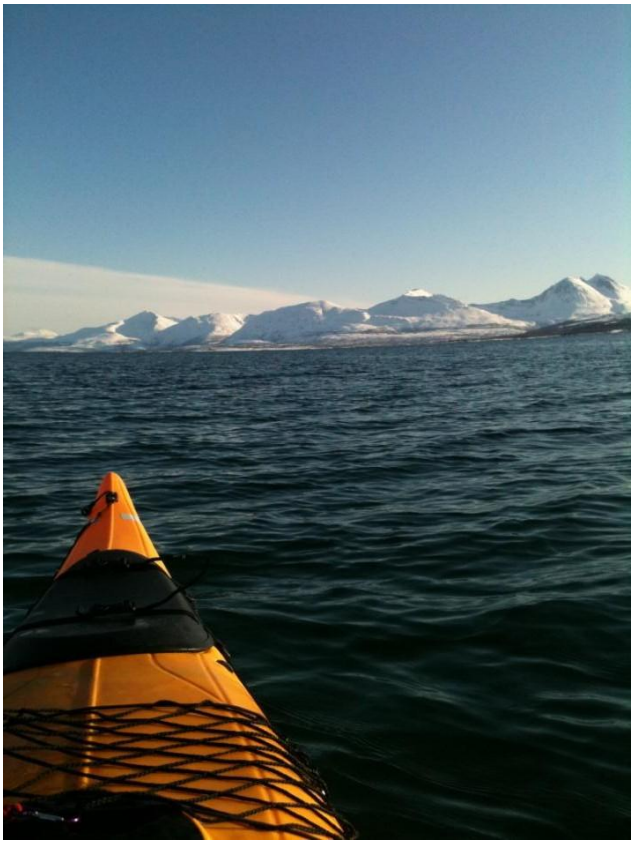
Validation of two different methods to determine cardiac ventricular dimensions in rats during experimental hypothermia

5.årsoppgave i Stadium IV – Profesjonsstudiet i medisin ved Universitetet i Tromsø

Ingrid Aune Tveita, MK-07



Hovedveileder: Ganesh Acharya, MD, PhD, FRCOG

Biveileder: Timofei Kondratiev, MD, PhD

Tromsø, våren 2012

Sammendrag

Bakgrunn

Prosjektet kom i gang som en forstudie til en større studie der vi ønsker å undersøke varighet og nivå av terapeutisk hypotermi som hjerneprotektiv behandling av asfyktiske, nyfødte små forsøksdyr. I slike forsøksprotokoller er det ønskelig å ha en ikke-invasiv metode for måling av hjertefunksjon, spesielt under nedkjøling, for å vurdere sirkulatorisk toleranse/overlevelse i små forsøksdyr.

Målet med denne studien var å teste ut om ekkokardiografisk metode er en pålitelig og anvendbar undersøkelsesteknikk ved å sammenligne venstre ventrikkels dynamiske volumendringer (endring i slagvolum, SV) under forskjellige korpstemperatur målt ved hjelp av ekkokardiografi (ikke-invasiv metode) og Millar konduktanskateter (invasiv gullstandard metode).

Material og metode

Forsøkene ble utført på avdeling for komparativ medisin, UIT, august 2011. Totalt 8 rotter ble brukt som forsøksdyr. En rotte døde under instrumentering. Tre ble brukt som normoterme kontroller og 4 dyr ble gradvis kjølt til 15^0C (angis som hypoterme). Tidssynkrone målinger av venstre ventrikkels dynamiske volumendringer i systole og diastole ble gjort med de to ovennevnte metoder. Fra de målte dimensjoner beregnet man slagvolum (SV) fra ligningen: SV = endediastolisk volum (EDV) – endesystolisk volum (ESV). Totalt 55 parvise SV- målinger ble gjort, og disse data har blitt brukt i den statistiske analysen.

Resultater

Vi fant at SV-målingene utført med ekkokardiografi var signifikant høyere enn de målt med Millar konduktanskateter (Related-Samples Wilcoxon Signed Rank Test $p < 0.0001$), men det var en signifikant (Spearman`s rho = 0.43; $p = 0.001$) sammenheng mellom SV-målinger utført med disse to metoder. Bland-Altmann analyse viste at gjennomsnittsdifferansen mellom SV målt med invasiv og non-invasiv metode var 103 uL

(95% konfidensintervall, 26.75 – 179.27 uL). Vi gjorde også den observasjon at SV under hypotermi øker gradvis ved kjøling ned til 25^0C, for så å avta noe ved 20^0C and 15^0C. Under måling med ekkokardiografi observerte vi i noen hypoterme rotter en dramatisk reduksjon i endesystoliske dimensjoner, med en tilnærmet fullstendig tømning av venstre ventrikkel (VV).

Fortolkning

VV SV målt med ekkokardiografi er signifikant høyere, men korrelerer godt med SV målt med gull-standard invasiv metode (Millar konduktanskateter) hos rotte. Vi har verifisert at ekkokardiografisk metode er en pålitelig og anvendbar metode for måling av dynamiske endringer i hjertevolum ved induksjon av hypotermi i små dyr, hele veien fra 37^0C til 15^0C.

Content

Acknowledgements

I wish to thank Professor Ganesh Acharya, Women´s Health & Perinatology Research Group, University of Tromsø, who introduced me to the clinical challenge of newborn asphyxia and the potential method of treating these newborns by applying therapeutic hypothermia. This has been of great inspiration to me. Further I want to thank him for introducing me to echocardiography and for patiently assisting in conducting the measurements during experiments. He also guided me through the procedure of evaluating LV-measurements from tracings obtained during experiments.

I also wish to express my gratitude to Timofei Kondratiev, MD, PhD, Senior Engineer at the Anesthesia and Critical Care Research Group, for performing the surgical procedures and conducting the placement of the conductance catheter in the left ventricle of the experimental animals. He also guided me through sampling of left ventricular functional data by use of the conductance catheter, and provided me with valuable background information for describing the method.

Introduction

This project was originally initiated due to the recognition that therapeutic hypothermia might have a protective role on cerebral function in the asphyxiated newborns [1-4]. Therapeutic hypothermia is already established as a cerebro-protective method after cardiac arrest in adult patients. One crucial question would be the temperature tolerance in the healthy and asphyxiated newborns. During literature search we were excited to find that in newborn animals hypothermia tolerance is well established. In ancient literature [8] we found that the temperature tolerance in the newborn fetus with respect to circulatory stability is way above that of the adult. Thus, it is plausible that the increased temperature tolerance among newborns is not solely related to heart and circulatory function but also to cerebral function.

One important part of evolution in mammals appears linked to tolerance of the fetus to physical stresses during and shortly after delivery. It is known that newborn hearts tolerate asphyxia and hypercapnic acidosis [5], ischemia-reperfusion [6], and even endotoxemia [7] better than the adult hearts. In general the newborn heart is equipped with slow skeleton troponin-I (ssTnI) which is being replaced by cardiac troponin I (cTnI) after weeks, depending on species. Myofilaments of neonatal hearts expressing ssTnI are more sensitive to cytosolic Ca^{2+} than the adult heart expressing cTnI. Likewise, hypothermia may be regarded a physical threat during birth, and at least shortly after delivery. It has long been known that newborn mammals, which constitutionally express ssTnI in their hearts, survive low body temperatures that cannot be tolerated by adults of the same species [8]. In 1948 Fairchild [9] reported that intraperitoneal temperature in rats from birth up to 17.5 days of age depends largely on environmental temperature, i.e. newborn rodents are poikilothermic. Further, she reported that infant rats (<17 days of age) survived in vivo rewarming from cardio-pulmonary arrest at 5ºC for 1 to 2 hours. For comparison, in adult rats cessation of spontaneous heartbeat is reported to take place during cooling to 16-12.5ºC. Interestingly, when these experiments were repeated using

in vitro techniques these relations with respect to temperature tolerance of the newborn heart could not be found, i.e. the increased tolerance of the newborn heart to hypothermic cardiac arrest in vitro was not different from the adult heart [10]. Therefore, it appears important to study differences in tolerance to hypothermia using *in vivo* models.

In the adult patient therapeutic cerebral protective effects following resuscitation from cardiac arrest are achieved by maintaining core temperature at 32 – 34^{0}C for 24 hours. The level and duration of induced hypothermia in adult survivors of cardiac arrest is more limited to circulatory tolerance at reduced core temperature than to the optimal level and effects of hypothermia on cerebral survival. However, it remains to determine if by increasing level of hypothermia and/or duration of hypothermia one may achieve a better brain protection in asphyxiated newborns who appear to have a much better circulatory tolerance to reduced core temperatures than adults. A suitable experimental animal model to initiate such investigations could be a Guinea pig model. However, due to the small size of the newborn heart a non-invasive method is needed to monitor cardiac function during induced therapeutic hypothermia. Echocardiography appears to be a suitable non-invasive method, but it remains to be determined if this is a reliable method to investigate cardiac function in the hypothermic heart. In order to monitor cardiac function during hypothermia, a collaborating research group at our University has been using an invasive method, the left ventricular (LV) pressure volume conductance catheter. This method is presently the gold standard for hemodynamic monitoring, but it is invasive and temperature dependent.

Thus, the objective of our present experimental study was to compare the non-invasive echocardiographic method for measuring LV volume, with the "gold standard" invasive conductance catheter technique at different temperatures. This was done to validate the use of non-invasive method for monitoring cardiac function of the asphyxiated fetus/neonate following establishment of induced hypothermia to achieve cerebral protection in small animals under experimental conditions.

Materials and methods

Experimental protocol was approved by the Norwegian Animal Research Authority (Project ID 3579). Animals were obtained from Harlan UK Limited, England. All procedures and care were in accordance with the Guide for the Care and Use of laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA).

A total of 8 animals (male Wistar rats, weighing 250 – 350g) were used. One rat died during instrumentation. Three of them were used as normothermic controls and not cooled. Hypothermia was induced in four animals. The measurements of LV dimensions were performed at temperature levels of 37, 30, 25, 20 and 15^{o}C.

Anesthesia. Anesthesia was induced by sodium pentobarbital (50 mg/kg body wt i.p.), followed by a continuous infusion of 7.5 mg/kg/h through an i.v. line in the left femoral vein. This infusion was discontinued on cooling, since hypothermia has anesthetic effects and decreases drug metabolism. The animals were monitored for any sign of discomfort during hypothermia and rewarming so that an additional anesthesia might be provided if necessary.

Core cooling. As described in previous studies [11-14], the animals were cooled by circulating cold water through U-shaped polyethylene tubes placed in the lower bowels. The tubes were gently introduced rectally, and care was taken not to harm the intestine. In addition, the double-layered operating table made of hollow aluminum was circulated with temperature-adjusted water. Core temperature was continuously monitored using a thermocouple wire inserted into the esophagus, whose wire was connected to a thermocouple controller (Thermoalert, Columbus Instruments, Columbus, OH) (Figure 1).

Respiratory Support. The rats were placed on the operating table in a supine position. The trachea was opened and a small-size metal tube (13G) was inserted for an external ventilator. All animals had spontaneous and sufficient ventilation at core temperatures > 20^{o}C.

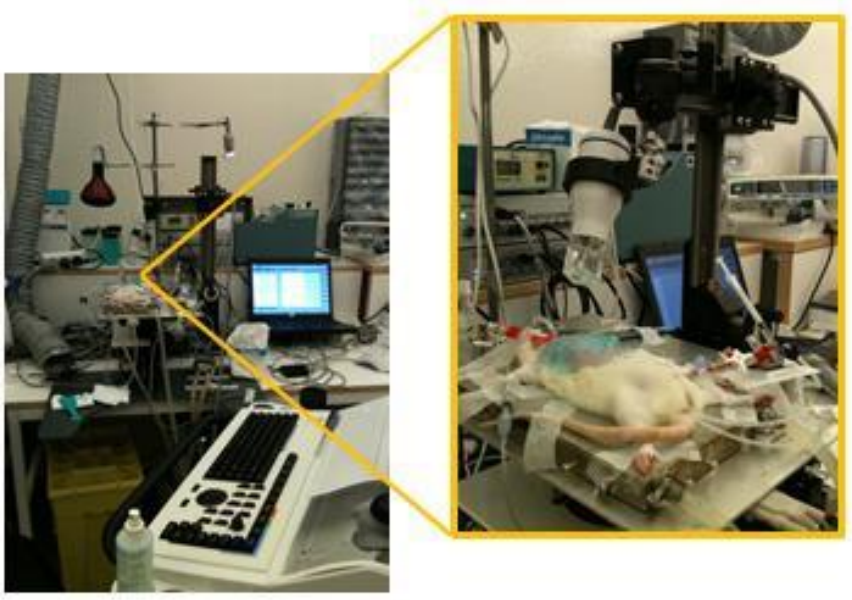


Figure 1. Experimental set up in the laboratory demonstrating anesthetized instrumented rat on a double-layered operating table made of hollow aluminum circulated by temperature- adjusted water ready for high-resolution echocardiography.

Hemodynamic measurements. Hemodynamic variables were obtained using the Millar pressure-volume conductance catheter system (SPR-838, Millar Instruments Inc, Texas), which has been utilized in prior studies [14-16]. The pressure-volume conductance catheter was inserted into the left ventricle via the right carotid artery. The miniaturized 2-French pressure-volume conductance catheter allowed for the assessment of *in vivo* LV function in rats [14]. The conductance catheter had four electrodes (E1, E2, E3, and E4). The spacing between E2 and E3 was 9.0 mm. In that space was a 2.0 F pressure transducer. A constant current to the blood inside the left ventricle was supplied by the two outer electrodes (E1, E4) and voltage changes in the electrical field were sensed by the two inner electrodes (E2, E3). Thus, the measured electrical potential was inversely in proportion to the conductance of blood surrounding the catheter in the left

ventricular chamber on the basis of an Ohm's law. A constant sinusoidal alternating current (0.02 mA root means square at 20 kHz) was applied to drive the conductance. The measured conductance should be corrected for parallel conductance induced by the alternating current passing through the blood into the surrounding ventricular structures or interventricular septum. A saline bolus injection method is generally used to measure parallel conductance at the end of experiment. However, this method was not applied in this study due to the multiple experimental temperatures (37, 30, 25, 20 and 15^{o}C) of which measurements were taken. The viscosity of blood is affected by temperature. Due to the lack of calibration of each temperature by the saline bolus that would have a fatal effect on the animal, the volume measurements in this study included parallel conductance (G_p). Since the pressure transducer was located between the conductance electrodes, measurement of pressure and conductance of blood surrounding the catheter in the left ventricle could be recorded simultaneously. In addition, mean arterial pressure (MAP) was measured using a pressure transducer connected to a fluid-filled catheter (22G) inserted into the left femoral artery. This MAP reflects peripheral vascular responses to cooling and rewarming.

In vitro volume cuvette calibration at different temperatures. For a more accurate assessment of the left ventricular volume, the cuvette calibration was performed using insulator-type cuvettes of known diameter (2, 3, 4, 5, 6, 7 mm) filled with heparin-treated blood. The volume measured by the conductance method inserting a four-electrode catheter (SPR-838) into the volume cuvette was calculated by the following formula: the actual volume between electrodes (E2-E3) is $\pi r^2 L$ where r is radius of the cuvette and L (9 mm) the distance between E2 and E3. The volume cuvette containing heparin-treated blood was placed on the inside of a thermo-controlled water circulator so that the temperature of the blood could be adjusted during the calibration. Considering temperature-dependent viscosity of blood, the volumes measured at our specific experimental temperature (37, 30, 25, 20 and 15^{o}C) were corrected using the cuvette calibration method. Linear regression at each temperature are presented in Figure 2.

Slopes and y-intercepts determined on linear regression at the different temperatures were applied to convert conductance units (relative volume unit: RVU, 1 RVU= 75 μMho) to true volume units (μl).

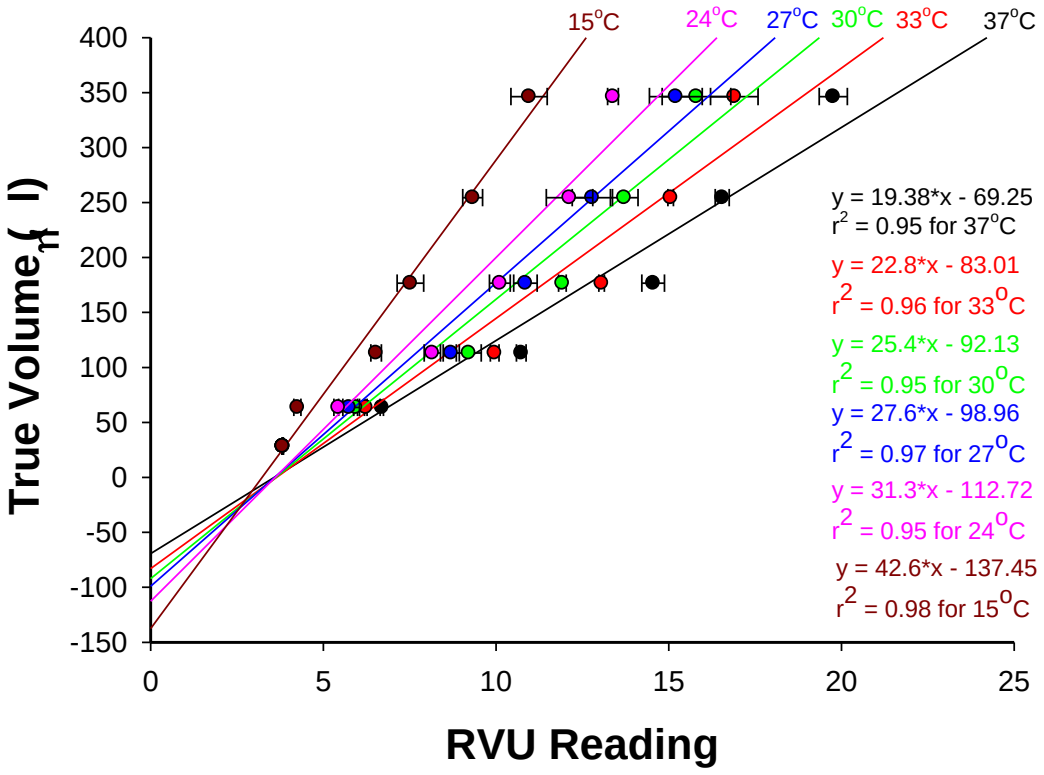


Figure 2. Regression analysis showing temperature dependent changes in relative volume units (RVU) – values vs. true volume at each temperature (Figure by the courtesy of Timofei Kondratiev, MD, PhD).

Echocardiography. Transthoracic echocardiography was performed using a high resolution ultrasound imaging system equipped with a RMV-710B transducer with a frequency of 25 MHz, and a fixed focal length of 15 mm (Vevo 770, Visualsonics, Toronto, Canada). Hair was removed with a mechanical shaver and application of depilatory cream and prewarmed ultrasound gel was applied to the skin. M-mode recordings were obtained from the parasternal short-axis views. All ultrasound measurements were performed off-line. The internal dimensions of the left ventricular (LV) cavity were measured during end-diastole and end-systole. From these measurements LV volumes were automatically determined by the machine software. The LV end-diastolic volume (EDV) was calculated as 7.0/(2.4 + LV IDd) x LV IDd3 and the LV end-systolic volume (ESV) was calculated as 7.0/(2.4 + LV IDs) x LV IDs3 assuming spherical shape of the left ventricle, where IDd is internal diameter during end-diastole and IDs is internal diameter during end-systole. The LV stroke volume (SV) was calculated as EDV – ESV.

Experimental protocol. Following surgery, the animals were allowed to rest for 60 minutes to achieve stable condition prior to hemodynamic measurements. The LV dimensions by M-mode echocardiography and pressure-volume loops by Millar conductance catheter were recorded simultaneously at normothermic condition (37ºC), and during hypothermia at 30, 25, 20, 15°C. A total of 55 pair-wise measurements where used for comparisons. Two separate operators acquired the M-mode echocardiographic cine loops and the conductance catheter derived pressure-volume loops simultaneously, while a command to start and stop recording was given by a third person.

Data acquisition. LV pressure and volume signals were digitized at 1 kHz, and recorded by ADInstruments Chart DAQ software. The ADInstruments Chart DAQ software allows the raw pressure and volume data to be on display in a scrolling strip chart format and also to be plotted against each other in real-time on the online XY plot window so that the pressure-volume loops can be continuously monitored, during which data of interest can be saved on a computer hard disk on request. The recorded raw data were analyzed off-line

using a Millar Pressure Volume Analysis (PVAN) software providing various hemodynamic parameters including SV.

Statistical analysis. Data were analyzed using PASW (Predictive Analytics SoftWare) Statistics version 18 (SPSS inc. Chicago, IL, USA). Related-samples Wilcoxon signed rank test was used to compare differences between LV stroke volumes measured by echocardiography and Millar conductance catheter, taking into account the repeated measurement design of the study and temperature differences. Correlation between SV measurements obtained using two different methods was tested using nonparametric Spearman `s rank correlation, and the agreement between these measurements was investigated using Bland-Altman analysis.

Results

We found that the SV measured by M-mode echocardiography was significantly higher than the SV measured using Millar conductance catheter (Related-Samples Wilcoxon Signed Rank Test $p < 0.0001$) (table 1), but there was a significant correlation between SV measurements performed by these two methods (Spearman`s rho = 0.43; p = 0.001).

Table 1. Left ventricular stroke volume (SV) measured using Millar conductance catheter and M-mode echocardiography in Wistar rats at normothermia and during hypothermia at different temperatures.

Temperature (^{0}C)	**37**	**30**	**25**	**20**	**15**
Mean SV Millar catheter (uL)	40.85	53.65	61.94	57.45	36.00
Mean SV Echocardiography (uL)	127.27	160.11	178.53	164.84	141.85
Number of observations	11	7	5	7	25

Bland-Altman analysis for limits of agreement showed a mean difference of 103 uL (95% CI, 26.75-179.27 uL) in SV measurement between using these two methods (Figure 3).

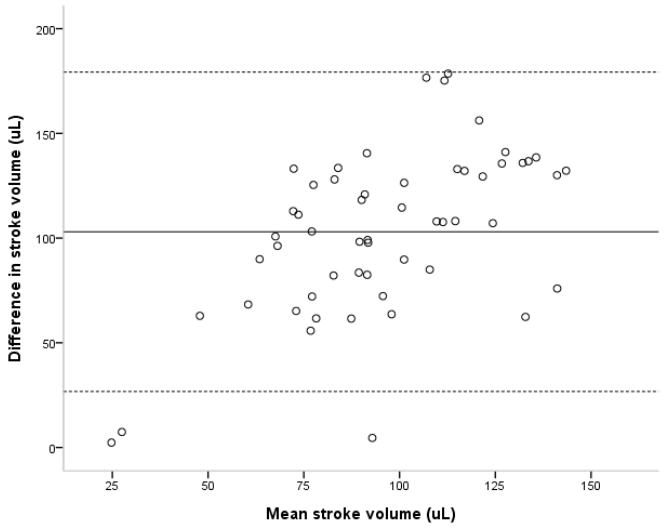


Figure 3. Bland-Altman plot of the difference versus the mean of paired measurements demonstrating agreement between simultaneously measured stroke volumes in rats at different temperatures using gold standard invasive (pressure volume conductance catheter) and noninvasive (M-mode echocardiography) techniques.

We also observed that during hypothermia the SV increased gradually by cooling until 25^0C and then reduced slightly at 20^0C and 15^0C. During echocardiography in some hypothermic rats, we observed a dramatic reduction in end-systolic dimension with an almost complete emptying of LV (Figure 4).

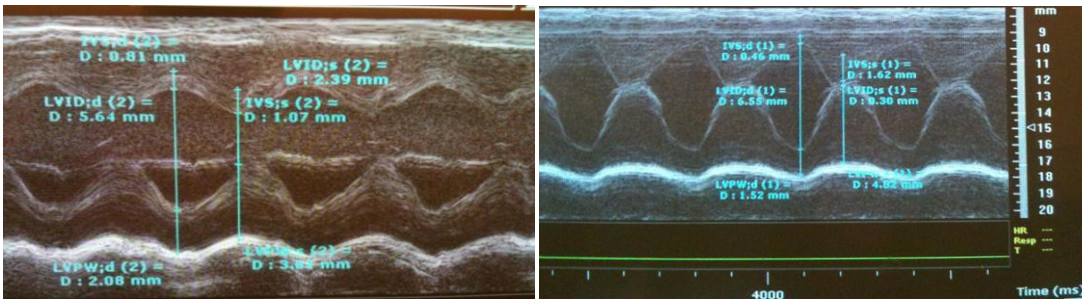


Figure 4. M-mode echocardiogram of a rat during normothermia (left) and hypothermia (right) demonstrating a dramatic reduction in end-systolic dimension of the left ventricular cavity .

Discussion

During cooling we know that 1) dynamic changes in LV volumes takes place as a consequence of changes in core temperature, and 2) a temperature-dependent change in electric conductance of blood takes place and influences the conductance measurement method. This last aspect is already taken care of in the present experiment as this particular change in electrical conductance is adjusted for during change in core temperature in order to present reliable, temperature-independent changes. The present experimental study demonstrates that there is a statistically significant correlation in volume determination when comparing the invasive LV pressure-volume conductance catheter and the non-invasive echocardiographic methods. This implies that echocardiography is a suitable non-invasive method for monitoring dynamic changes in cardiac function of small animals following establishment of induced hypothermia. Non-invasive method can be particularly useful for repeated measurements to assess serial changes in stroke volume. However, we found significant differences in SV in terms of absolute values measured by two techniques and the limits of agreement were wide. Therefore, the precision and accuracy of SV measurement can be questioned.

Another important practical aspect related to potential limitation of using trans-thoracic echocardiography during hypothermia has been ruled out by the present experiment. Despite hypothermia-induced changes in LV anatomical configuration, which is an important factor that may interfere with LV volume determination, reliable measurements of LV volumes can be achieved during cooling down to 15ºC.
The volume estimations done by means of echocardiographic measurements were temperature independent, while the volumes obtained by conductance catheter were adjusted for temperature. The significant correlations between the two different methods at all temperatures supports the notion that the corrections made to adjust for temperature-induced changes in conductance are likely to be correct.

Measurement of dynamic changes in LV volumes in small animals is a complicated technical procedure. Previously cardiac output (CO) measurements in small

animals have been made by means of thermodilution techniques and radiolabelled microspheres. By means of these methods CO estimations have been conducted in small animals and SV has been calculated by dividing CO with heart rate (HR). However, by using microspheres the numbers of CO measurements during experiments were limited to the number of isotopes at hand. These methods gave reliable and reproductive measurements of CO and SV, but when comparing CO measurements and SV determinations made by these two different methods, the volumes varied considerably. Even between experiments using the same technique these variables varied. Therefore, it has been mandatory before these techniques could be used in experimental series for determining CO that reproducibility could be documented. As a consequence, to document relative changes in these variables during experiments have been taken as prove of physiologic changes rather than comparing with standard physiologic values as often used in human medicine. One of the reasons for the differences in SV estimations in small animals has been considered due to the fact that HR in these animals are out of proportion high (400 to 500 beats/min) and thus determination of SV may depend on animal strain and the type of anesthesia used. Therefore, by comparing LV volume measurements using the two different methods in the present experiment it was not expected to measure exactly the same LV volumes in µl in synchronized heart beats. More interesting, when comparing the two methods, was to compare changes in these variables as achieved by changing temperature and thus LV volumes in this animal model. By comparing a new method (echocardiography) with an already established method (conductance catheter measurements) only such comparisons will indicate if the new method is reliable and applicable in the present setting.

Another interesting aspect that turned out during the experiment was that the SV in rodents increases during cooling as demonstrated by dramatic reduction in the LV internal dimension and almost complete emptying of LV during end- systole. Although not part of the experimental protocol, due to its general novelty as a not yet well explored physiologic phenomenon, background physiologic information to explain this observation is included.

A hypothermia-induced increase in SV has been documented by several investigators using isolated cardiac muscle preparations or isolated heart preparations. However, to the best of our knowledge the present experiment is the first to document the extent of LV emptying in rodents in vivo as a consequence of induced hypothermia which implies a ejection fraction of close to 100% .This finding is clearly demonstrated by fig. 4. The hypothermia-induced increase in peak force or developed force of isolated hearts or myocardial muscle tissue in vitro has long been proposed due to the elevated intracellular Ca2+ levels (Ca2+)i. The increase in (Ca2+)i, may be caused by the following mechanisms: A temperature-dependent slowing of the Na+/K+ pump activity [18] induces subsequent increases in intracellular sodium level (Na+)i [19,20]. This increased (Na+)i will retard Ca2+ extrusion through the Na+/Ca2+ exchange system, more Ca2+ can be taken up into sarcoplasmatic reticulum, and released during subsequent beats. Another mechanism which may increase (Ca2+)i during hypothermia, and thereby increase developed force, is a reduced Ca2+ extrusion through the Na+/Ca2+ exchanger in late systole and during diastole [21]. Reported influence of hypothermia on systolic function using intact models or isolated preparations is controversial [22]. However, this historical controversy is mainly due to the limited methods at hand at the time this controversy was created. As already stated, the present experiment is the first to show this almost complete emptying of the LV as a consequence of deep hypothermia. Some investigators have found decreased isovolumetric peak in intact models during hypothermia [23,24], but this decrease was thought to be the consequence of anesthesia and drugs. However, later works from in vivo canine models in which the anesthetized animal was cooled for several hours, indexes of systolic function were depressed. In contrast, studies of excised cross-circulated hearts have demonstrated an increase in Emax, a load-independent measure of contractility derived from systolic pressure-volume relations [25-28]. These studies have observed that, although E_{max} increases with cooling, the time to maximal time- varying elastance (T_{max}) also increases [25]. This finding contrasts with the effects of catecholamine stimulation, in which E_{max} increases but T_{max} decreases. From these isolated heart studies, it appears that cooling

increases the strength of contraction but may prolong its duration [29]. The net effect of increased E_{max} and prolonged T_{max} may still be decreased ability to produce useful work. An increased calcium uptake or a prolonged systolic calcium elevation has been proposed as important for the observed changes of contractile function in hypothermia [13,30].

Calcium overload, demonstrated under in vitro conditions [13], is probably a characteristic of the beating heart during moderate and deep hypothermia. Another theory for the increase in SV is that prolongation of the diastolic period during hypothermia permits better filling of the heart and brings about an increased stroke volume. Both LV dP/dtmax and LVSP were decreased at these temperatures in rats. The results concerning changes in contractility in response to cooling are in accordance with studies on rat hearts in vitro [31]. In isolated preparations, the peak force increases, whereas the rate of rise as well as the relaxation rate decreases. This could be due to a change in intracellular calcium homeostasis. In support of this theory is the finding that at normal temperatures, ouabain (a sodium pump inhibitor) augments the left ventricular contractile capacity, while in hypothermia its effect appears less effective, and even depressive effects of digitalis on the hypothermic myocardium have been reported [32]. Later studies have considered that the increase in contractility of canine purkinje fibers at 35ºC was mainly due to an increase in Ca^{2+} sensitivity of myofibrils [33]. An increase in Ca^{2+} sensitivity was proposed to be responsible for the hypothermia-induced ventricular fibrillation as early as in 1957 by Angelakos et al. [34]. Newer studies have shown that, by means of phosphprous-31-nuclear magnetic resonance spectroscopy, during mild hypothermia, increase in myocardial inotropy of isolated rabbit hearts was not related to intracellular pH or energy-relate phosphorus compounds, but to a primary change in myofilament Ca^{2+} responsiveness [35]. Further, one mechanism by which hearts of cold-blooded animals (trout) is able to maintain contractility at low temperatures (0ºC) are not due to changes in activity of myofibrillar ATPase activity, but thorough the inherent higher Ca^{2+} sensitivity of the contractile elements compared to mammals (rat) [36]. This finding is in accordance with the

observation that a very profound cooling always decreases the contractile forces of mammalian heart [37,38] and decreases the velocity of contraction [39].

In the present experiment we find the typically hypothermia-induced increase in SV as outlined above. We have measured this increase in SV as being due to an increase in left ventricular emptying (measured as reduced end-systolic volume) rather than being due to a change in left ventricular filling volume (measured as maintained end-diastolic volume).

Conclusion

Left ventricular stroke volume measured by noninvasive M-mode echocardiography in rats at different body temperatures was significantly higher than that measured by Millar conductance catheter technique, but there was significant correlation between these two methods. The echocardiographic method appears to be reliable and useful for monitoring dynamic changes in LV volume during hypothermia (down to 15^0C) in small animals.

Reference List

1. Westin B, Enhorning G: **An experimental study of the human fetus with special reference to asphyxia neonatorum.** *Acta Paediatr Suppl* 1955, **44:**79-81.

2. Gunn AJ, Gluckman PD, Gunn TR: **Selective head cooling in newborn infants after perinatal asphyxia: a safety study.** *Pediatrics* 1998, **102:**885-892.

3. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgins RD et al.: **Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy.** *N Engl J Med* 2005, **353:**1574-1584.

4. Higgins RD, Shankaran S: **Hypothermia: novel approaches for premature infants.** *Early Hum Dev* 2011, **87 Suppl 1:**S17-S18.

5. Urboniene D, Dias FA, Pena JR, Walker LA, Solaro RJ, Wolska BM: **Expression of slow skeletal troponin I in adult mouse heart helps to maintain the left ventricular systolic function during respiratory hypercapnia.** *Circ Res* 2005, **97:**70-77.

6. Arteaga GM, Warren CM, Milutinovic S, Martin AF, Solaro RJ: **Specific enhancement of sarcomeric response to Ca2+ protects murine myocardium against ischemia-reperfusion dysfunction.** *Am J Physiol Heart Circ Physiol* 2005, **289:**H2183-H2192.

7. Layland J, Cave AC, Warren C, Grieve DJ, Sparks E, Kentish JC, Solaro RJ, Shah AM: **Protection against endotoxemia-induced contractile dysfunction in mice with cardiac-specific expression of slow skeletal troponin I.** *FASEB J* 2005, **19:**1137-1139.

8. Edwards W.F.: *Influence des Agens Physiques Sur la Vie.* Paris: Crochard; 1824.

9. Fairfield J: **Effects of cold on infant rats.** *Fed Proc* 1948, **7:**32.

10. Adolph EF: **Responses to hypothermia in several species of infant mammals.** *Am J Physiol* 1951, **166:**75-91.

11. Kondratiev TV, Tveita T: **Effects of sympathetic stimulation during cooling on hypothermic as well as posthypothermic hemodynamic function.** *Can J Physiol Pharmacol* 2006, **84:**985-991.

12. Kondratiev TV, Flemming K, Myhre ES, Sovershaev MA, Tveita T: **Is oxygen supply a limiting factor for survival during rewarming from profound hypothermia?** *Am J Physiol Heart Circ Physiol* 2006,
291:H441-H450.

13. Kondratiev TV, Wold RM, Aasum E, Tveita T: **Myocardial mechanical dysfunction and calcium overload following rewarming from experimental hypothermia in vivo.** *Cryobiology* 2008, **56:**15-21.

14. Han YS, Tveita T, Kondratiev TV, Prakash YS, Sieck GC: **Changes in cardiovascular beta-adrenoceptor responses during hypothermia.** *Cryobiology* 2008, **57:**246-250.

15. Filseth OM, How OJ, Kondratiev T, Gamst TM, Sager G, Tveita T: **Changes in cardiovascular effects of dopamine in response to graded hypothermia in vivo.** *Crit Care Med* 2012, **40:**178-186.

16. Filseth OM, How OJ, Kondratiev T, Gamst TM, Tveita T: **Post-hypothermic cardiac left ventricular systolic dysfunction after rewarming in an intact pig model.** *Crit Care* 2010, **14:**R211.

18. Eisner DA, Lederer WJ: **Characterization of the electrogenic sodium pump in cardiac Purkinje fibres.** *J Physiol* 1980, **303:**441-474.

19. Bers DM: **Ryanodine and the calcium content of cardiac SR assessed by caffeine and rapid cooling contractures.** *Am J Physiol* 1987, **253:**C408-C415.

20. Shattock MJ, Bers DM: **Inotropic response to hypothermia and the temperature-dependence of ryanodine action in isolated rabbit and rat ventricular muscle: implications for excitation- contraction coupling.** *Circ Res* 1987, **61:**761-771.

21. Sutko JL, Bers DM, Reeves JP: **Postrest inotropy in rabbit ventricle: Na+-Ca2+ exchange determines sarcoplasmic reticulum Ca2+ content.** *Am J Physiol* 1986, **250:**H654-H661.

22. Goldberg LI: **Effects of hypothermia on contractility of the intact dog heart.** *Am J Physiol* 1958, **194:**92-98.

23. Angelakos ET, HEGNAUER AH: **Pharmacological agents for the control of spontaneous ventricular fibrillation under progressive hypothermia.** *J Pharmacol Exp Ther* 1959, **127:**137-145.

24. Angelakos ET: **Influence of pharmacological agents on spontaneous and surgically induced hypothermic ventricular fibrillation.** *Ann N Y Acad Sci* 1959, **80:**351-364.

25. Suga H, Sagawa K: **Instantaneous pressure-volume relationships and their ratio in the excised, supported canine left ventricle.** *Circ Res* 1974, **35:**117-126.

26. Suga H, Abe M: **Myocardial contractility of right and left ventricles of variously weighted mammals.**
Johns Hopkins Med J 1973, **132:**227-233.

27. Suga H, Sagawa K, Shoukas AA: **Load independence of the instantaneous pressure-volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio.** *Circ Res* 1973, **32:**314-322.

28. Suga H: **Left ventricular time-varying pressure-volume ratio in systole as an index of myocardial inotropism.** *Jpn Heart J* 1971, **12:**153-160.

29. Suga H, Sagawa K: **Mathematical interrelationship between instantaneous ventricular pressure-volume ratio and myocardial force-velocity relation.** *Ann Biomed Eng* 1972, **1:**160-181.

30. Steigen TK, Tveita T, Hevroy O, Andreasen TV, Larsen TS: **Glucose and fatty acid oxidation by the in situ dog heart during experimental cooling and rewarming.** *Ann Thorac Surg* 1998, **65:**1235-1240.

31. Steigen TK, Myrmel T, Aasum E, Larsen TS: **Enhancement of Hypothermia Induced Rise in Myocardial Calcium Content by Fatty-Acids.** *J Mol Cell Cardiol* 1992, **24:**S97.

32. Konishi T, Apstein CS: **Deleterious effects of digitalis on newborn rabbit myocardium after simulated cardiac surgery.** *J Thorac Cardiovasc Surg* 1991, **101:**337-341.

33. Sprung J, Stowe DF, Kampine JP, Bosnjak ZJ: **Hypothermia modifies anesthetic effect on contractile force and Ca2+ transients in cardiac Purkinje fibers.** *Am J Physiol* 1994, **267:**H725-33.

34. Angelakos ET, LAFORET EG, HEGNAUER AH: **Ventricular excitability and refractoriness in the hypothermic dog.** *Am J Physiol* 1957, **189:**591-595.

35. Kusuoka H, Ikoma Y, Futaki S, Suga H, Kitabatake A, Kamada T, Inoue M: **Positive inotropism in hypothermia partially depends on an increase in maximal Ca(2+)-activated force.** *Am J Physiol* 1991, **261:**H1005-H1010.

36. Churcott CS, Moyes CD, Bressler BH, Baldwin KM, Tibbits GF: **Temperature and pH effects on Ca-2+ sensitivity of cardiac myofibrils: A comparison of trout with mammals.** *American Journal of Physiology* 1994, **267:**-R70.

37. Bernard A, Hunter JE, Fuller BJ, Imoedemhe D, Curtis P, Jackson A: **Fertilization and embryonic development of human oocytes after cooling.** *Human Reproduction* 1992, **7:**1447-1450.

38. Monroe RG, STRANG RH, LaFarge CG, LEVY J: **Ventricular performance, pressure - volume relationships, and O2 consumption during hypothermia.** *Am J Physiol* 1964, **206:**67-73.

39. Kurihara S, Sakai T: **Effect of rapid cooling on toad and guinea pig cardiac muscles.** *Recent Adv Stud*

Cardiac Struct Metab 1976, **11:**181-184.

The students contribution to the project

Etter flere innledende planleggingsmøter startet prosjektet opp 8. august 2011.

Forsøkene ble utført på avdeling for komparativ medisin, UIT , august 2011.

Kandidaten deltok i planleggingen av prosjektet og var med på alle forsøkene som ble utført.

Kandidaten fikk innføring i, og deltok under instrumeniteringen av forsøksdyrene. Kandidaten fikk videre opplæring i hjertefunskjonsmåling både vha. Millarkateter (invasivt konduktanskateter) og Ekkokardiografi (non-invasiv) av hovedveileder Dr. Ganesh Acharya.

Kandidaten deltok i alle målingene som ble utført.

Kandidaten var med på å bearbeide resultatene innhentet fra både Millar- og Ekko-monitorering og fikk innføring i statistisk metode for så å lage grafiske framstillinger av dette.

Kandidaten har iløa våren 2012 skrevet sammen resultatene til oppgaven i sin nåværende form.