Effect of acute body positional changes on the haemodynamics of rats with and without myocardial infarction

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In humans, the lateral recumbent position has a beneficial effect on haemodynamics. If this effect is substantial in small animals too, there is a risk of misinterpretation in preclinical investigations. Therefore, the aim of this study was to analyse the impact of acute changes in body position on haemodynamics in rats. Healthy rats (n = 21) and rats post myocardial infarction (n = 20) were randomly positioned supine, prone, or on the right or left side. In each position, we measured haemodynamic parameters by pressure-tip catheter and thermodilution. We found that left ventricular contractility (dP/dt max) was significantly elevated in both lateral positions as compared to the supine position in healthy rats. In healthy rats and following infarction, cardiac index (CI) and stroke volume index (SVI) were significantly higher in both lateral positions as compared to the supine or prone position. Of importance, if SVI values in the supine position in healthy rats (0.095 ± 0.003 ml (100 g)⁻¹) are compared to SVI values measured in different positions after myocardial infarction, the SVI can be either significantly lower in the supine (0.084 ± 0.003 ml (100 g)⁻¹) or significantly higher in the left lateral position (0.105 ± 0.003 ml (100 g)⁻¹). We conclude that post myocardial infarction and in healthy control rats, important haemodynamic values are increased in lateral positions as compared to prone or supine positions. Analysing haemodynamic data in rats may therefore result in misinterpretation if the body position is inconsistent.

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A beneficial effect of lateral recumbent positions was initially reported in the 1930s (Wood & Wolferth, 1935) in patients suffering from cardiac disease. More recently, newer methods of haemodynamic measurements stimulated anaesthesiologists and intensive care physicians to put effort in clarifying the impact of body positioning on global haemodynamics. It appeared, that cardiac function is enhanced in the lateral positions during laparoscopic surgery (Fujise et al. 1998), lumbar spine operations (Yokoyama et al. 1991) and during the stay in the intensive care unit following major surgery (Doering & Dracup, 1988). The right lateral position was also recently confirmed to improve haemodynamics in patients suffering from heart failure (Fujita et al. 2002). However, other human analyses failed to demonstrate any significant difference regarding the cardiac performance when the body is rotated to recumbent positions other than dorsal (Lange et al. 1988; Yokoyama et al. 2000; Pump et al. 2002). In addition, the physiological mechanism of these possible haemodynamic variations is not fully understood.

Most preclinical studies involve animal research, especially for the analysis of new therapies with possible haemodynamic effect. Thus, the impact of body positioning needs to be verified as significant haemodynamic variations may lead to different interpretations of the study. Indeed, the lateral position was actually confirmed to increase both cardiac output and blood pressure in large-animal models (Nakao et al. 1986; Bornscheuer et al. 1996). But the effect of body positional changes was never clearly reported in
small-animal models. In the present study we therefore hypothesize, that lateral positioning as compared to prone or supine positioning increases cardiac output and filling pressure parameters in normal rats as well as in rats after coronary ligation and subsequent myocardial infarction.

**Methods**

**Animal model**

The animals received human care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health. All procedures were performed in accordance with the Swiss Animal Protection Law after obtaining the permission of the State Veterinary Office, Bern, Switzerland.

For all procedures male Wistar rats were anaesthetized with isoflurane (5% oxygen for induction and 2.5% oxygen for maintenance) and supplemental buprenorphine (10 µg kg⁻¹ subcutaneously). We placed the rats on a warming pad (37°C) and intubated the animals orally with subsequent positive pressure ventilation at 80 cycles min⁻¹ (14-gauge i.v. cannula, AbboCath, Abbott, Sligo, Republic of Ireland; Small Animal Ventilator 683, Harvard Apparatus Inc., Holliston, MA, USA). The tip of the intubation cannula was placed in the trachea, a safe distance proximal to the bifurcation, and then fixed in place by ligature to avoid dislodging the cannula and single lung ventilation when changing body position.

One group of healthy rats received no special treatment before the haemodynamic investigations. In the second group of animals, a myocardial infarction was induced 3 weeks before the haemodynamic measurements. Briefly, through a left lateral thoracotomy, the left anterior descending artery was ligated at the outflow of the first diagonal branch using a 7/0 polypropylene suture (Ethicon Inc., Somerville, NJ, USA).

**Animal instrumentation**

We positioned cutaneous ECG needles on the four limbs. After shaving and midline cervical incision, the right jugular vein and carotid artery were exposed. Both a 1.4-French pressure microcatheter (Millar, Mikro-Tip, Millar Instruments, Houston, TX, USA) and a thermocouple probe (MLT1405, ADInstruments, Castle Hill, Australia) were inserted in the right carotid artery. A security suture was then placed around the two catheters and the artery to fix the catheters in place and effect haemostasis.

The tip of the Millar catheter was positioned in the left ventricle (LV), whereas the tip of the temperature probe remained positioned in the ascending aorta. Data were recorded using the PowerLab system (Chart 5.2, PowerLab, ADInstruments). Finally, a 20-gauge catheter was inserted through the jugular vein and its tip placed into the right atrium for future bolus saline injection (thermodilution method for cardiac output measurement, see below). All catheters were fixed in place to prevent dislocation during the experimental protocol. The catheter position for haemodynamic measurement is presented in Fig. 1.

**Haemodynamic measurements**

In a random order, we alternatively placed the animals in supine, prone, right lateral and left lateral position. The animals were allowed 5 min for stabilization in each body position before haemodynamic data were recorded.

![Figure 1. Catheter setting for haemodynamic measurements](image-url)

The tip of the Millar catheter is placed in the left ventricle (LV). For cardiac index measurement by thermodilution method, we injected a cold saline bolus of 0.1 ml through the catheter in right atrium (RA). The temperature probe will record the variation of temperature in the aortic arch.
We do not present the absolute LV pressure values because the transmural pressures could not be measured. As all pressures are measured relatively to ambient pressure, one cannot assume that they correctly reflect the intraventricular distension pressures, in particular when the body position and thus the gravitational load on the transducers are changed. Moreover, because the pressure catheters are very sensitive over time, especially when the body position and thus the gravitational load on the transducers are changed. Consequently, we decided not to rely on the absolute pressure values but rather on the developed LV pressure, defined as the difference between the minimal value measured during a cycle. We therefore express the developed systolic LV pressure as the difference between this minimal value and the maximal systolic LV pressure (dLVP$_{sys}$). Similarly, the end-diastolic pressure (EDP) is expressed as the difference between the EDP and the minimal value obtained during the cycle (dLVEDP).

From an arbitrary chosen group of 10 subsequent cycles, we calculated the mean values for dLVP$_{sys}$, dLVEDP, heart rate (HR), LV contractility (dP/dt$_{max}$) and relaxation (dP/dt$_{min}$). Before and after the measurements we calibrated the Millar catheter by zeroing in a 37°C blood drop and by correcting systematic pressure changes in the transducer.

The cardiac index (CI) was measured using the thermodilution method (modified for rats) as described previously (Cabras et al. 2003). Briefly, a bolus of 0.1 ml ice cold 0.9% saline was injected into the right atrium through the jugular vein catheter. CI was calculated using the following formula:

$$CI = \frac{k_{spec} \times (T_{rat} - T_{inj}) \times (V_{inj} - V_{dead})}{\int_{0}^{\Delta T(t)} \cdot dr \times BW}$$

where $T_{rat}$ represents the rat body temperature, $T_{inj}$ is the injectate temperature (4°C), BW is the body weight, $V_{inj}$ is the injectate volume (0.1 ml), $V_{dead}$ is the dead space volume of the venous catheter (0.02 ml), $\int_{0}^{\Delta T(t)} \cdot dr$ is the integral of temperature variation during an interval of 14 s, and $k_{spec}$ is the density factor defined as the product of specific heat and specific gravity of saline divided by the same product for blood ($k_{spec} = 1.102$ for 0.9% saline) (Loer et al. 1999). We did not make any correction for the potential heat exchange during the transit through the lung as it was previously shown that this loss of thermal energy through the lung tissue is negligible and that the total amount of the injectate is retrieved in the systemic side after one passage though the pulmonary vasculature (Arfors et al. 1971; Bock et al. 1988).

Dividing CI by HR represents the stroke volume index (SVI). All measurements were performed in triplicate for each body positioning.

### Infarct size measurement

After the animals were killed by potassium injection, we harvested the hearts and they were frozen, wrapped in a clear food wrap, for 24 h at −20°C. The frozen hearts were then cut orthogonal to the heart axis in 2.5-mm thick slices. We stained the slices with 1% triphenyl-tetrazolium-chloride (TTC) in phosphate buffer for 20 min in a shanking bath at 37°C and then fixed them during 20 min in 4% paraformaldehyde. Living tissues were then coloured in red whereas the infarcted areas remained pale in colour (Ytrehus et al. 1994). The slices were then compressed in a Plexiglass chamber and photographed with a digital camera. Using an image-analysis program (ImageTool 2.0, Microsoft Corporation, Redmond, WA, USA), we calculated the living and infarcted areas, then integrated the values of all slices to estimate the infarction size of three or four slices.

### Statistics

We carried out computer-assisted statistical analysis using SigmaStat (SPSS Inc. Chicago, IL, USA). The results are expressed as mean ± s.e.m. One-way ANOVA for repeated measurements assessed differences between body positions within a group. Unpaired t test revealed differences between single parameters from healthy and infarcted rats. If significant differences were detected, pairwise multiple comparison procedures (Holm-Sidak method) specified analysis. $P < 0.05$ was considered statistically significant.

### Results

#### Healthy rat hearts

For the healthy rats (n = 21, weight, 275 ± 34 g), the mean total experimental time was 105 ± 35 min. The injection of cold saline for cardiac output measurements resulted in an arterial temperature decrease from 36.1 ± 0.7°C to 35.5 ± 0.8°C during the procedure. No correlation was found between body position and temperature.

All haemodynamic data are presented in Table 1. Except for HR and dLVEDP, which appeared stable in all positions, the lowest values were obtained in the supine position. By contrast, the highest values were measured in the left lateral position. In fact, in both lateral positions – either left or right – CI, SVI, dP/dt$_{max}$, dP/dt$_{min}$ and dLVP$_{sys}$ were significantly increased as compared to the values obtained in either supine or prone posture. Moreover, the values for CI, SVI and dP/dt$_{max}$ were significantly higher in the left lateral position as compared to the right lateral position. In the prone position, only dP/dt$_{max}$ was significantly elevated as compared to the supine position.

The relative differences between the supine and other body postures are highlighted in Fig. 2A. We observed a
consistent progressive improvement of all haemodynamic parameters (except HR) when the body position was changed from supine successively to prone, right side and finally left side. The highest increases in haemodynamic parameters were indeed obtained in the left lateral position with a significant relative augmentation of the CI (19 ± 3%), SVI (21 ± 4%), dP/dt max (25 ± 5%), dP/dt min (23 ± 5%), and dLVP sys (17 ± 3%).

Table 1. Haemodynamic parameters in the healthy rat heart (n = 21)

<table>
<thead>
<tr>
<th>Position</th>
<th>CI (ml min⁻¹ (100 g⁻¹))</th>
<th>SVI (ml (100 g⁻¹))</th>
<th>HR (beats min⁻¹)</th>
<th>dP/dt max (mmHg s⁻¹)</th>
<th>dP/dt min (mmHg s⁻¹)</th>
<th>dLVP sys (mmHg)</th>
<th>dLVEDP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supine</td>
<td>31.4 ± 1.0</td>
<td>0.095 ± 0.003</td>
<td>334 ± 10</td>
<td>3387 ± 197</td>
<td>−3001 ± 193</td>
<td>86.1 ± 3.7</td>
<td>3.80 ± 0.17</td>
</tr>
<tr>
<td>Prone</td>
<td>32.9 ± 1.1</td>
<td>0.099 ± 0.003</td>
<td>332 ± 9</td>
<td>3661 ± 184*</td>
<td>−3216 ± 164</td>
<td>90.6 ± 3.6</td>
<td>3.78 ± 0.23</td>
</tr>
<tr>
<td>Right</td>
<td>34.6 ± 1.1*§</td>
<td>0.105 ± 0.003*§</td>
<td>332 ± 10</td>
<td>3912 ± 186*§</td>
<td>−3401 ± 207*</td>
<td>95.2 ± 4.1*§</td>
<td>3.77 ± 0.25</td>
</tr>
<tr>
<td>Left</td>
<td>36.9 ± 1.0*§#</td>
<td>0.113 ± 0.004*§#</td>
<td>330 ± 10</td>
<td>4097 ± 165*§</td>
<td>−3559 ± 172*§</td>
<td>98.2 ± 3.2*§</td>
<td>3.95 ± 0.24</td>
</tr>
<tr>
<td>Mean</td>
<td>33.9 ± 0.9</td>
<td>0.103 ± 0.003</td>
<td>332 ± 10</td>
<td>3764 ± 174</td>
<td>−3294 ± 170</td>
<td>92.5 ± 3.4</td>
<td>3.86 ± 0.19</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.; *P < 0.05 versus supine, §P < 0.05 versus prone, #P < 0.05 versus right side. CI, Cardiac index; SVI, stroke volume index; HR, heart rate; dP/dt max, LV contractility; dP/dt min, LV relaxation; dLVP sys, developed systolic LV pressure; dLVEDP, developed LV end-diastolic pressure.

Infarcted rat hearts

Myocardial infarction was produced in 43 rats as previously described. With this method, 22 animals (51%) died within the first 24 h due to cardiac decompensation or arrhythmia. One rat was excluded later because the infarction size was too small. Thus, we included 20 rats (weight, 304 ± 37 g) with myocardial infarctions in the study.

We measured haemodynamic parameters 3 weeks after coronary ligation. The mean total experimental time was 113 ± 24 min. The injection of cold saline for cardiac output measurements resulted in a temperature decrease from 36.03 ± 0.8°C to 35.3 ± 0.9°C during the procedure. No correlation was found between body position and temperature. The infarction size confirmed by TTC staining was 19.8 ± 5.5% of the LV.

All haemodynamic data are presented in Table 2. As for normal rats, HR and dLVEDP remained stable in every position. Similarly too, the highest haemodynamic values were observed on the left lateral side whereas the lowest values were obtained when the animals were placed supine or prone. In both lateral positions – either left or right – CI, SVI, dP/dt max and dLVP sys were significantly elevated as compared to values obtained in the prone position. When compared to values measured in the supine position, only CI and SVI were significantly higher. Moreover, the values for CI and SVI were also significantly higher in the left lateral position as compared to the right lateral position. In the prone position, CI and SVI were significantly elevated compared to the supine position. However, dP/dt min was not significantly altered by the variation in body positioning.

The percentage differences between the supine and other positions are highlighted in Fig. 2B. We observed a maximal relative augmentation of the haemodynamic parameters in the left lateral position with a significant increase for CI (21 ± 2%), SVI (23 ± 2%) and dLVP sys (7 ± 2%).

Comparison of healthy and infarcted rat hearts

The infarcted hearts developed a significant increase in dLVEDP. Three weeks after myocardial infarction, the values for dP/dt max, dP/dt min and dLVP sys were unchanged as compared to the same positions in normal hearts. However, the SVI after infarction was reduced (Fig. 3A). Nevertheless, the increased HR allowed compensation of the CI which appeared thus similar in healthy rats and following myocardial infarction. Two important observations can be now reported. Firstly, the statistically significant SVI reduction observed in the supine position in infarcted hearts as compared to normal hearts (0.084 ± 0.003 versus 0.095 ± 0.003 ml (100 g)⁻¹), P < 0.05) was abolished when measurements were taken in the right lateral position (0.098 ± 0.003 versus 0.105 ± 0.003 ml (100 g)⁻¹, n.s.) (Fig. 3A).

Secondly, as highlighted in Fig. 3B, the various SVI values obtained after myocardial infarction can be either significantly lower, or not significantly different, or even significantly higher if compared to the single supine position in healthy control hearts.

Discussion

In rats with a moderate myocardial infarction as well as in healthy rats, haemodynamics perform better in both lateral positions as compared to prone or supine positions. In healthy rats, we confirm a large increase of up to 25% for important haemodynamic parameters such as CI, SVI, dP/dt max, dP/dt min and dLVP sys when the body position is switched from supine to the left lateral side. Following myocardial infarction, positional changes also influence cardiac performance, but less markedly. Nevertheless, a more than 20% increase of CI
and SVI values could be obtained in the lateral position as compared to supine position. It is interesting that the output (CI), pressure (dLVP$_{sys}$) and contractility (dP/dt$_{max}$, dP/dt$_{min}$) parameters are equal to those in healthy hearts, but a significantly decreased SVI is compensated by an elevated HR. Besides, the left ventricular preload parameter dLVEDP is increased in the diseased compared to the healthy hearts. Therefore, the moderate myocardial infarction (20% of LV) leads to a compensated heart failure.

The improved venous blood drainage towards the right heart is frequently proposed to explain the better haemodynamic performance achieved when the body is placed on the left side. Indeed, the diameter of the inferior vena cava as well as the right atrial blood pressure was reported to be larger in humans positioned on their right side (Nakao et al. 1987). When lying on the back, the weight of the organs, and specially the abdominal organs, may compress the inferior vena cava with subsequent reduction of venous blood return to the heart. Another potential mechanism involves the autonomic nervous system and its effect on the systemic vascular resistance (Fujise et al. 1998; Kuo & Chen, 1998; Fujita et al. 2000; Miyamoto et al. 2001). In the current study, we did not specifically assess the central venous pressure (CVP), and therefore we are not able to verify these possible explanations in the rat model. Besides the inability to calculate systemic vascular resistance without CVP, this value would also allow a better evaluation of the mechanisms involved in positional haemodynamic changes after myocardial infarction. Also, the ligation of one carotid artery means that one baroreflex region was unloaded and

![Figure 2. Relative variations in haemodynamic parameters](image)

Relative variations (% as compared to the supine position) of the parameters measured in healthy ($A; n = 21$) and in infarcted rat hearts ($B; n = 20$). *$P < 0.05$ versus supine position in the same group, †$P < 0.05$ versus prone position in the same group, ‡$P < 0.05$ versus right position in the same group.
this may equally disturb background conditions in all positions.

Obviously the supine position is not physiological in almost all species. One could thus naturally speculate that the haemodynamic performance would be altered in this position as compared to more physiological body positioning. However, surprisingly the haemodynamic parameters measured in the current study in the most physiological prone position, although better than in the supine position, are still inferior as compared to both lateral positions.

The purpose of our study was, however, not to determine the physiological mechanisms explaining those position-dependent haemodynamic variations. We rather wanted to confirm that these variations are not only limited to humans and large-animal models but that they affect small-animal models as well. The rat model is typically used in cardiovascular research either to understand the mechanisms of cardiovascular disease or to assess the potential benefit of new therapeutic modalities. The results of these studies may significantly influence further investigations and, especially, evaluations of new therapies as the results of the animal research are important before initiating clinical studies. For this purpose, the value of animal analyses is essential. Our current analysis highlights the fact that a simple body positioning may significantly affect the outcome. Indeed a variation of up to 25% of certain haemodynamic parameters could be observed by simply rotating the rat from the supine position to its left lateral side. But more importantly we showed that the comparison of haemodynamic values measured between control healthy rats and rats with moderate heart failure could easily be misinterpreted. As a clear example, we demonstrated that the SVI in rats after myocardial infarction could either be significantly decreased, not significantly altered, or even significantly increased as compared to a normal and healthy group of rats (Fig. 3B). In consequence, we suggest that haemodynamic parameters should be consistently recorded in the same position in all groups involved in a study, and the position of data acquisition needs to be reported as well. The observation that haemodynamic parameters are higher on the side as compared to the prone position makes it difficult to define the ‘correct’ position in which haemodynamic parameters should be recorded. The prone position is the most physiological position in rats. However, the values obtained in the left lateral position most suitably reflect the absolute values published in other studies with anaesthetized rats (Conzen et al. 1992; Yang et al. 1995; Vogel, 1997; Moreau et al. 1998; Sharp & La Regin, 1998; Pacher et al. 2004). In addition, it is more convenient to place the catheterized animals on the side as compared to the prone position. Therefore, we recommend measuring haemodynamics in anaesthetized rats in the left lateral position, even though this parameter can obviously not mirror haemodynamics in the conscious rat.

The present study has some limitations. Firstly, we analysed a group of animals in the early phase of heart failure development. Rats with a moderate myocardial infarction were, 3 weeks after coronary ligation, still able to compensate for the myocardial loss. Contractility and relaxation as well as CI were not significantly altered as compared to normal non-ligated animals placed in the same body position. However, the dLVEDP was consistently augmented in all positions which confirms the loss of LV compliance. In addition, the SVI was also significantly reduced. Rats with advanced heart failure are, however, very unstable and therefore long recording procedures such as the one used in this protocol may be hazardous. Mortality would also have been much higher.

A second limitation of the current study may be that one cannot trust the absolute pressure values obtained in the present setting. This may be due to a changing gravitational load on the transducer while the animals are moved from one position to the other and the subsequent unknown transmural pressure, and especially due to a deviation of the zero line over the duration of the procedure (Kingma et al. 1996). The values for dLVEDP and dLVPys could, however, be easily calculated and compared between the groups. Finally, we calculated the SVI and CI using the thermodilution method. This technique was recently validated in small animals (Cabrales et al. 2003). We found that this approach was relatively easy and especially reproducible if the injection catheter in the right atrium is placed and fixed correctly and if the injectate volume
is defined precisely. The limitation of this technique may be the undetected heat exchange during the passage through the pulmonary vasculature. The CI and SVI may consequently be overestimated, but it has been reported previously, that the heat exchange in the lungs is negligible (Arfors et al. 1971; Bock et al. 1988; Loer et al. 1999). In addition, this potential error was reproduced systematically in all of our experiments so that a valid comparison could be made between the positions.

In conclusion, the recording of haemodynamic data is highly influenced by the body position in healthy rats as well as in rats 3 weeks after coronary ligation.

**Figure 3. Comparison of stroke volume index**

Stroke volume index in healthy (A) and infarcted (B) rat hearts. *P < 0.05 versus supine position in the same group, †P < 0.05 versus prone position in the same group, #P < 0.05 versus right position in the same group, ‡P < 0.05 versus same position in healthy rats.
The left lateral posture provides the best and most physiological haemodynamic data and is therefore the recommended position for haemodynamic measurements in rats. Because of the differences, results of studies comparing various groups of animals can easily be misinterpreted if the body position under experimental conditions is not consistently applied and taken into account.

References


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