



SUMMARY AND CONCLUSIONS

In the present thesis we have investigated heterogeneous local blood flow in the left ventricle of the heart, and compared this to local oxygen consumption measured in the same tissue sample at the same time point. For many years it has been known that blood flow can be very heterogeneous, but the relation with local oxygen demand was unclear. From previous studies it was known that there was likely an adaptation of local supply to demand in the heart, but because oxygen consumption could not be measured at sufficiently small spatial resolution at the same time as oxygen delivery, this point was not proven. In all studies described in this thesis, we compared oxygen consumption per heart estimated by NMR spectroscopy to oxygen consumption as calculated from blood gas data to validate our method. In all studies a rather good correlation was found, which, in our opinion, at least means that the NMR-based method is quite robust.

A disadvantage of the method is the destructive character. In our experiments the animal was sacrificed because it was necessary to freeze-clamp the heart at the end of the experiment, in order to stop metabolism as quickly as possible. Further, we can only estimate local oxygen consumption at one time point, i.e. at the end of the experiment, while we can measure local blood flow at various moments. However, in the control group, blood flow was quite stable over time. With other techniques, especially positron emission tomography, it might be possible to get the same results, but the resolution is still less than for our method. Better spatial resolution with our ^{13}C method may be possible if metabolites are measured with mass spectrometry.

Discrepancy between different groups may be caused by experimental procedures. Basal blood flow in the control group was lower than in the ischemia group before occlusion was applied. A possible reason might be that we manipulated the left anterior descending artery by applying a partial occluder around the artery, causing a difference in blood flow distal to the occluder. However, we have no reason to believe that blood flow heterogeneity is different in this group.

We know that measurement of blood flow by microspheres has relative errors of about 5%, and the ^{13}C NMR method has even larger errors. For the NMR method the error margins depend on the parameters that are analysed. For the estimation

of myocardial oxygen consumption only two parameters are necessary, i.e. the speed of TCA cycle flux, and the rate of incorporation of ^{13}C in the TCA cycle. For these parameters, measurement errors are between 10 and 20 %. When other flux parameters are estimated, error rates can increase substantially, and depend on the intervention per experiment. When standard deviations of TCA cycle flux reached a certain threshold value, samples were discarded from the analysis.

In some experiments, NMR spectra were of lower quality than in other experiments. This might be related to the lower rate of incorporation of ^{13}C in the intracellular metabolite pools, for example in the ischemia groups. In these groups some samples had to be excluded because of too low quality of the spectra. For some spectra only two or three NMR peaks could be analysed, making estimation of oxygen consumption from these samples impossible. The sometimes low quality of the spectra also causes oxygen extraction rates higher than 1, which must be the result of measurement error. However, in many tissue samples the quality of the spectra was good, and analysis was straightforward. Nevertheless, these first measurements of local aerobic metabolism in tissue samples were still far from ideal and improvement of methodology and measured spectra are highly desirable.

NMR spectroscopy is only one of the methods to analyse ^{13}C incorporation into the TCA cycle. Another method would be mass spectrometry, which, theoretically, might give less noisy results or allow much smaller tissue samples to be analysed. We have performed some tests to see whether mass spectrometry can be performed in our tissue sample extracts, and the results were promising. However, we only have very preliminary data, and this method needs to be evaluated in the future. Another interesting feature for the future might be to correlate local blood flow and oxygen consumption to local myocardial work. This might be done by echocardiography or by magnetic resonance imaging, in order to view cardiac motion directly. Translating these motions into reliable measures of cardiac work is usually not really straightforward and measurement of oxygen consumption aligned with the images would be challenging.

During the time-course of performing experiments and analysing the data, three different computational methods were used. For the control group as described

in Chapter 3 we used the method as described earlier in the article by Van Beek et al. (*Am J Physiol Heart Circ Physiol* 277: H1630-1640, 1999). However, it was difficult to estimate the auxiliary parameters with this method, especially for NMR spectra of low quality, and the analysis was very time-consuming. Therefore we developed a different method to estimate parameters in a difficult metabolic system such as the tricarboxylic acid cycle. The new method includes an enhanced parameter optimisation strategy (named FluxEs), and includes error estimation of the quantitated fluxes and possible incorporation of prior knowledge via Bayesian priors. The software package allows assembling the model equations for arbitrary metabolic systems using a simple input format. This avoids extensive computer programming to hard code the metabolic pathways and experimental protocols. By using this method only a rough estimate of parameter confidence regions was obtained by assuming local linearity.

Until now we used fixed values for the parameters transport time, anaplerotic flux, and exchange rate. These values we derived from model fits in which these parameters could be reasonably estimated. However, the assumption that these parameters are similar in all experimental conditions might not be true. We wanted to improve this method by introducing a Markov chain Monte Carlo (MCMC) parameter estimation procedure which allows a full description of the confidence regions of the estimated metabolic fluxes, including correlations and nonlinear dependencies between parameter estimates. We also took into account the measurement error of the NMR spectra and uncertainty of model parameters.

The figures below show the correlation between oxygen consumption data derived from the first (Van Beek et al, 1999, Alders et al, 2004; details described in Chapter 3) and second (Binsl et al., 2011) method and data from the second and third (Hettling et al., 2013; details described in Chapter 7) method. Despite the increased sophistication of method 2 which used priors and allowed limited variation of some auxiliary parameters, its results tended to correspond with those of method 1. Method 3 gave higher values for oxygen consumption in a large subset of the samples. The reason is mainly that in Method 3 the value for the anaplerotic flux is not constrained to low values, but is estimated with a quite broad prior. This causes higher values for the TCA cycle flux if estimates for the anaplerotic flux are high. (Note: method 1 was only used for the control group, hence the difference in number of samples.)

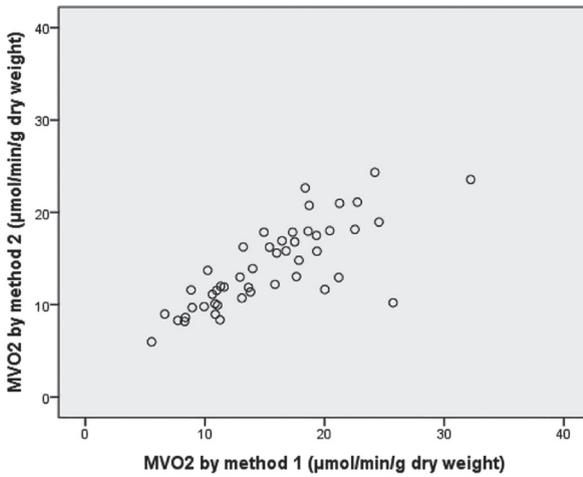


Fig. 1A. Myocardial oxygen consumption calculated by method 1 versus method 2 (described above). Data points are individual tissue samples from all hearts of the control group (Chapter 3)

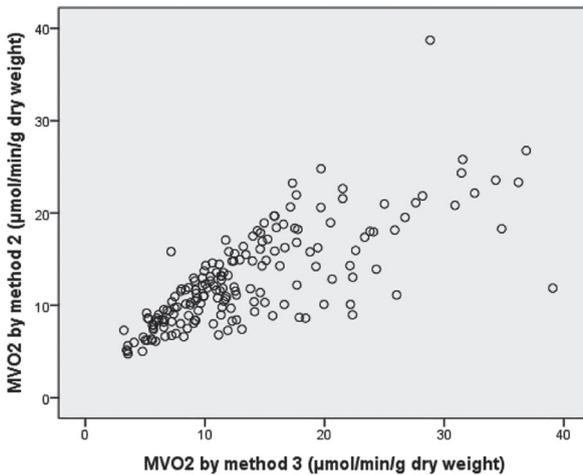


Fig. 1B. Myocardial oxygen consumption calculated by method 2 versus method 3 (described above). Data points are individual tissue samples from all hearts from the control group, the ischemia group and the LPS control group (Chapters 3, 5 and 6)

For all experimental groups and for all estimation methods, there was usually a good correlation between oxygen consumption measured from blood gas values, hemoglobin and blood flow and oxygen consumption estimated by the NMR method.

Examples of the correlation between the oxygen consumption measurements for the different methods are shown in Figure 5 of Chapter 3, Figure 1 of Chapter 6, and Figure 3 of Chapter 7.

We described possible explanations for heterogeneity of local blood flow and local oxygen metabolism. We showed that heterogeneity of blood flow can, at least partially, be explained by heterogeneity in oxygen demand. The degree of heterogeneity was basically the same in innervated and chronically denervated heart tissue. However, probably by causing vasoconstriction, innervation led to a small decrease in blood flow, for the same local oxygen consumption. During graded stenosis of the left anterior descending coronary artery, we found that relative heterogeneity of oxygen delivery increases. Blood flow and oxygen delivery were redistributed, and correlation of oxygen supply to demand decreased progressively. Also, during decreased myocardial function due to endotoxic shock we demonstrated a mismatch between oxygen delivery and oxygen consumption. We found that the ratio between local oxygen uptake and local oxygen delivery was often decreased, suggesting local overperfusion or reduced oxygen uptake.

In the introduction we speculated on the possibility to improve the function of cardiac tissue that has been damaged by ischemia. Indeed we found that by infusion of a vasodilator such as adenosine the relation between oxygen delivery and oxygen consumption was improved, possibly by increasing blood flow to damaged heart areas. Therefore, we speculate that such an intervention might be beneficial to the ischemic heart.

In the following paragraphs, the chapters in this thesis are briefly summarised:

As reviewed in **Chapter 2**, various methods exist to measure local blood flow, especially in heart tissue. The method which produces the most accurate measurements in small tissue samples are labelled microspheres of about 15 μm \emptyset , but all methods show that regional blood flow is quite heterogeneous. Also, in whole hearts, positron emission tomography shows the same amount of heterogeneity of blood flow, but the resolution is still too low to use in small tissue samples. Oxygen metabolism can also be measured by various methods. We have developed a model of ^{13}C incorporation into the tricarboxylic acid

cycle to calculate local oxygen consumption in small tissue samples taken from the heart *in situ*. In this chapter we briefly describe the method, and how oxygen consumption and blood flow are related in normal hearts.

In **Chapter 3** we applied this method to pig hearts, in which no pharmacological intervention was done. The pigs were anaesthetized, intubated and instrumented. At two time points local blood flow was measured with radioactive microspheres, showing relatively stable perfusion over time. During the experiment ^{13}C labelled acetate was infused into the left anterior descending artery, leading to incorporation of ^{13}C into the TCA cycle, which has a fast exchange with glutamate. Because glutamate is present in relatively large quantities, this metabolite was used for the NMR measurements. At the end of the experiment part of the heart was freeze-clamped and processed as described in this chapter. We calculated oxygen consumption with the NMR method and compared it with measurements from blood samples. There was a significant correlation between the two measurements. Taking into consideration various sources of error for both measurements, we conclude that almost half of the variance of blood flow is explained by heterogeneity in oxygen demand.

In **Chapter 4** we investigated the effect of sympathetic innervation on local blood flow, its heterogeneity and the relation with oxygen consumption in chloralose-anaesthetised dogs. There was no significant difference in blood flow or heterogeneity of blood flow between the innervated and denervated areas, but in the denervated areas we found a slight increase in blood flow for each level of oxygen consumption. This implies a slight vasoconstrictive effect of sympathetic innervation on coronary blood vessels. Again, a significant relation was found between local oxygen delivery and oxygen demand.

Matching of local blood flow and oxygen consumption was measured in pigs with a partially stenosed left anterior descending (LAD) artery in **Chapter 5**. Two degrees of stenosis were applied to the artery, and in one stenosed group adenosine was infused as a potent vasodilator. The groups were compared with a control group. A significant relation between oxygen delivery and demand was found in the control group, but this relation was gradually lost in the stenosis groups. Infusion of adenosine partially improved the correlation again.

We found that regions which had relatively high blood flow before stenosis were not more metabolically vulnerable during partial coronary stenosis.

Lipopolysaccharide (LPS)-infused pigs were studied in **Chapter 6**, showing endotoxic shock. These animals were hypotensive compared to controls, but maintained cardiac output, blood flow and oxygen delivery. Regional blood flow was strongly redistributed during LPS infusion, and the correlation between oxygen delivery and oxygen uptake fell dramatically. This mismatch may be responsible for myocardial depression in sepsis, at least in part.

In the final **Chapter 7** we partially analysed the same data with a slightly different mathematical method. Much attention is given to the computational model analysis to estimate local oxygen consumption from the NMR spectra, with emphasis on five parameters for the citric acid cycle and related metabolism. Simulations were performed to analyse which parameters could be measured with modest error levels. Different priors (which roughly means value ranges known from previous studies) for the various parameters were simulated. For all metabolic states there was a relatively good correlation between oxygen consumption measured with the NMR method and derived from blood gas samples for the whole heart. We found that the two parameters necessary for flux estimation could be measured relatively well, but that auxiliary parameters could not be estimated properly with our relatively noisy NMR data.

In conclusion, we present methods to simultaneously measure local oxygen uptake and local oxygen delivery in pig and dog hearts. We applied this method to animals without any intervention and to animals subjected to regional denervation, ischemia, vasodilation, and sepsis. The method has its drawbacks, but for the first time we show that, at small sample size, local oxygen delivery is adapted to local oxygen consumption, although not perfectly, and assess how this adaptation is compromised during denervation, ischemia and endotoxic shock.

