CHAPTER 1

General introduction

To sustain the circulation of blood to the human body, the heart requires energy. To this end, the fuel and oxygen required by the heart muscle for its pumping action are supplied via the coronary blood vessels which pass through the ventricular wall. Coronary stenosis, ischemia and myocardial infarction are important components of heart disease. When local blood flow in a certain myocardial region is low relative to the demand for oxygen and nutrients, insufficient energy for contractile and electrical function can be generated. On the other hand, a luxuriously high level of local perfusion causes an unnecessary pressure drop in the coronary arteries and may therefore limit the possibilities to increase myocardial perfusion in other locations. A good match of local myocardial blood flow to metabolic demand therefore appears to be desirable.

It is well-known, that the perfusion of the cardiac muscle with blood is very heterogeneous, even in the healthy heart (1-3). The cause of this is not very clear. The relative decrease in blood flow in coronary stenosis is also strongly heterogeneous, particularly within the subendocardial and subepicardial muscle layers, although it depends on the experimental model used (4,5). A poor adjustment between the local blood supply and O_2 consumption can lead to arrhythmias, and often heterogeneous "spotwise" ischemia and necrosis in myocardial infarction. Blood flow heterogeneity has been a research subject for many years, and there were many speculations in order to explain this heterogeneity. Many researchers have performed experiments in which local blood was related to a surrogate measurement for oxygen consumption, such as substrate uptake, measurement of enzyme activities or ATP concentrations, or lactate as a marker of ischemia (6-9). Further, experiments have been done to match local blood flow to local contractile function, such as systolic wall thickening or segment shortening, measured by MRI (10).

There were no methods to analyse local blood flow and local oxygen consumption at sufficient spatial resolution at the same time point in larger animals, although positron emission tomography has been used successfully in rodents and other small animals (11). We have, however, developed a new technique to measure the flux in the citric acid cycle, and thus estimate the O_2 consumption, in small tissue samples using substrate (acetate, lactate)

labelled with the stable isotope ^{13}C (12). We infuse substrate labelled with ^{13}C for a predetermined exact time period into the left anterior descending artery in situ and freeze-clamp the part of the left ventricle perfused by this artery, after which this part is divided into small tissue samples. The ^{13}C label incorporation in metabolites through the citric acid cycle, particularly glutamate, can be measured with high resolution nuclear magnetic resonance (NMR) spectroscopy in extracts of the samples. The flux can be estimated from the multiplet structure of the NMR spectrum. The local O_2 consumption can then be calculated, and compared with the local blood flow which is measured concurrently with radioactively labelled microspheres. In this way, we have found a solution for the "classic" problem of how to measure local O_2 consumption and local blood flow at the same time. The principle of measuring local oxygen consumption with the ^{13}C method has been shown in isolated perfused rabbit hearts, and in these hearts, oxygen consumption measured with the NMR method correlated well with oxygen consumption measured from the coronary flow and O_2 content (12). In this thesis we will measure local blood flow and oxygen uptake at a spatial resolution slightly below 1 milliliter.

In most of our experiments we used pigs of about 30-35 kg. Pigs have the right cardiac size for *in* situ instrumentation, have a similar cardiac and coronary anatomy and physiology as humans, and can be quite easily kept (13). There is almost no collateral blood flow, just like in humans. We needed an experimental model of this size because it was necessary to infuse substrate into the left anterior descending artery, and because of the requirement of analysis of many tissue samples from different heart layers.

We will investigate the effect of the heterogeneous distribution of the blood flow on the local energy supply during various conditions, such as reduced blood supply or disturbed regulation in comparison with the baseline situation. If we demonstrate that heterogeneity of metabolism in relation to the blood flow at sub-milliliter spatial resolution plays an important role, then we can study the effects of therapeutic interventions on the metabolic heterogeneity, for example by pharmacological vasodilatation with adenosine. One of the long-term goals would be to save as much myocardial tissue as possible from damage due to ischemia. Also, other negative consequences of heterogeneous

adjustment between blood flow and metabolism could be counteracted using the information obtained. In summary, in this thesis we investigate how local oxygen uptake and perfusion are matched, although both are distributed heterogeneously.

As mentioned above it is largely unknown why blood flow heterogeneity exists, although it is a well-established phenomenon since the eighties of the last century. In **Chapter 2** the literature is reviewed regarding heterogeneity of blood flow. We describe the various methods to measure local blood flow and blood flow heterogeneity and attention is paid to proven contributing factors to this heterogeneity. We assess the existing methods to calculate oxygen consumption and describe our new ^{13}C NMR method. Further, we describe why local oxygen delivery relates, at least in part, to local metabolic rate.

Until recently it was not understood why heterogeneity of blood flow exists in the heart. Local blood flow can be measured using radioactive or fluorescent microspheres of about 15 μm diameter, but it was not possible to measure local oxygen consumption in very small size tissue samples of about 50-150 mg dry mass (1-2 mm in the transmural direction). In an earlier paper (12) we described a method to measure local oxygen consumption in small tissue samples in rabbit hearts, using infusion of ^{13}C labelled acetate into the heart for a specific period of time.

In **Chapter 3** we apply radioactive microspheres to measure local blood flow, and use the ^{13}C method in hearts under basal metabolic conditions in anaesthetised pigs to measure local oxygen consumption. We measured local blood flow at two time points in order to demonstrate stability of the model, while in the same tissue sample local oxygen consumption was calculated by computational model analysis of the incorporation of ^{13}C in glutamate via the tricarboxylic acid cycle during timed infusion of ^{13}C labelled acetate into the left anterior descending artery. In this way the relation between local oxygen delivery and local oxygen consumption could be analysed. We elaborate on error factors in the measurement of both parameters, and speculate on the physiological meaning of the results found. This chapter defines the basal state in hearts that were not exposed to extra stress beyond being an open chest model.

In **Chapter 4** we describe a study performed in dogs, in collaboration with colleagues in London, UK and Maastricht, the Netherlands. Sympathetic innervation may influence local blood flow by increasing vasoconstriction with noradrenaline (thus decreasing blood flow), and higher noradrenaline levels may also increase myocardial contractility, thereby increasing local oxygen consumption. Therefore, the influence of sympathetic denervation on the relation of oxygen delivery and oxygen consumption could be interesting. Denervation was performed by applying phenol to the circumflex artery and surrounding tissues. The animals were allowed to recover for 3-4 weeks, after which denervation was sufficient. After this period experiments were performed in a similar way to those described in chapter 3, except for the use of ^{13}C lactate as substrate. Innervated and denervated tissue are compared with respect to blood flow and oxygen consumption, and the relation between these parameters is analysed.

As mentioned above, local oxygen delivery and local oxygen consumption are relatively well matched in normal hearts. However, during coronary stenosis, the relationship may change. It was previously shown that after partially occluding the left anterior descending (LAD) artery, leading to perfusion pressures of about 40 mmHg, blood flow reduction was highly variable. Lactate, as an indicator of ischemia, increased to a similar degree in areas of high- and low flow before stenosis was applied. This suggested that regions with high and low resting flows in the normally perfused heart are equally susceptible to metabolism-perfusion mismatch resulting from coronary stenosis. In **Chapter 5** we applied, again in pigs, two grades of coronary stenosis to the LAD, aiming for perfusion pressures of about 35 and 70 mmHg, and measured oxygen consumption in the same tissue samples during this stenosis. We wanted to investigate whether blood flow to oxygen consumption matching was relatively well maintained during stenosis, or whether matching was disproportionally decreased in areas at risk. This would suggest uneven distribution of cardiac ischemic stress. In the lowest perfusion pressure group, we also infused adenosine, as a potent vasodilator. This could help to maintain oxygen supply through stenosed arteries, but may not reach all distal areas.

It is well-known that myocardial depression is an important feature of septic shock. In previous studies a mismatch in oxygen delivery and demand was

suspected, because blood flow heterogeneity is largely unchanged, but flow distribution is completely altered. This was combined with a reduced net lactate extraction, suggesting mismatch of metabolism and perfusion. However, myocardial oxygen consumption could not be measured on a sufficiently small level until recently. In **Chapter 6** we infused pigs with *E. coli* endotoxin, to induce a sepsis-like model, and measured local oxygen uptake and blood flow. The data were compared with a time-matched control group, while infusing saline.

Chapter 7 describes a computational study to analyse the noisy ^{13}C-NMR spectra to quantify local oxygen consumption. Here we investigate a more sophisticated optimization method to fit the NMR-derived data to the mathematical model. We describe the use of the model under various metabolic conditions, and pay attention to the use of prior knowledge for the different parameters to estimate. In particular, the new method makes it possible to estimate confidence intervals for the calculated fluxes.

In conclusion, in this thesis we analyse the relation between local blood supply and demand in the heart. We apply a method previously described in isolated perfused rabbit hearts, to *in situ* pig hearts, in order to estimate local oxygen uptake and oxygen delivery. We describe the various methods to analyse oxygen uptake and delivery, and the matching between them. Then, we compare our data from the control group, with no intervention, to the data from various conditions, such as sympathetic denervation, various degrees of ischemia, and sepsis. Finally, we describe an improved method to estimate various flux parameters using single cardiac biopsies.

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