Stellingen
behorende bij het proefschrift
Intramycocardial blood volume and oxygen exchange

1. De toename van het coronaire bloedvolume na vasodilatatie moet met name gezocht worden op het niveau van de kleinste bloedvaatjes en niet in de coronaire venen.
   Dit proefschrift, hoofdstuk 2.

2. Het geven van waarden voor vatvolumes en doorbloedingen heeft alleen zin als duidelijk vermeld wordt hoe genormaliseerd is naar de hoeveelheid weefsel.

3. In de literatuur worden twee fenomenen van coronaire regeling, namelijk autoregulatie en metabole regulatie, vaak afzonderlijk beschreven. Het is echter goed mogelijk dat autoregulatie een vorm van metabole regeling is: na een verandering van de perfusiedruk wordt de doorbloeding aangepast aan het onveranderde metabolisme.


5. Om verbeteringen in de behandeling van coronaire stenoses te bewerkstelligen, is het belangrijk dat men zich niet alleen richt op de ontwikkeling van nieuwe behandeltechnieken, maar dat vooral het herstelproces van de bloedvaten na een ingreep wordt onderzocht.

6. Bij signalen die overwegend periodiek lijken, maar soms worden afgewisseld door schijnbare constantheid, mag deze verandering van de vorm van het signaal niet zonder meer toegeschreven worden aan veranderde meetomstandigheden, maar moet rekening gehouden worden met een mogelijk chaotisch gedrag van het onderliggende proces.

7. Het lage percentage sterfgevallen door hart- en vaatziekten in de derde wereld komt niet door een gezondere levenswijze.
8. Het feit dat de kosten van een kat of hond als huisdier enkele malen groter zijn dan de financiële ondersteuning van een Foster Parents kind zou moeten leiden tot het besef dat meer steun aan hulpbehoevenden in de ontwikkelingslanden mogelijk is.
Bron: De Asielkrant, September 1993, Nederlandse Vereniging tot Bescherming van Dieren

9. Het gebruik van de titel doctorandus voor vrouwen met een doctoraaldiploma is grammaticaal onjuist en draagt niet bij aan een toename van het besef dat er veel vrouwen in de wetenschappelijke wereld werkzaam zijn.

10. Bij zweefvliegwedstrijden op dagen met sterke thermiek zijn zwaardere zweefvliegers in het voordeel ten opzichte van hun lichtere collega’s.

11. Het getuigt van veel gemakzucht en weinig moed, dat veel mensen die in geval van nood een bloedtransfusie willen krijgen niet de moeite nemen om bloed of plasma af te staan.

12. Hopelijk is de aandacht die op dit moment aan het milieu geschonken wordt geen modegril; mode is immers al een grote milieubelasting.

Delft, 7 juni 1994                                       Kitty van der Ploeg
Intramycocardial blood volume and oxygen exchange
Op de voorkant: Afgietsel van de coronaire vaten die door de hartspier lopen  
On the cover: Cast of the coronary circulation of the heart

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Intramyocardial blood volume
and
oxygen exchange

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus prof.ir K.F. Wakker,
in het openbaar te verdedigen ten overstaan van een commissie,
door het College van Dekanen aangewezen,
op dinsdag 7 juni 1994 te 10.30 uur,
door

Catharina Petronella Bernardina Van der Ploeg

geboren te Voorhout,
doctorandus in de biomedische wetenschappen
Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.
Voor mijn ouders
Voor Arie
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1 Introduction

1.1 General introduction

In mammals, flow of blood is necessary to supply organs and tissues with oxygen and nutrients and to dispose their waste products. Furthermore, heat and hormonal factors are distributed via blood flow. The driving force for the circulation of blood is generated by the heart. The heart is a hollow muscle, that is divided in two parts by the septum. The left and right part of the heart again are divided in an atrium and a ventricle, which are separated by heart valves allowing flow of blood from the atria to the ventricles but not retrogradely. By contracting periodically blood is pumped through the body continuously.

The circulation of blood through heart, lungs and body is illustrated in Figure 1.1. The right atrium collects blood from the organs and tissues of the

![Diagram of blood circulation](image)

**Figure 1.1** The circulation of blood through heart, lungs and body. Adapted from Guyton (1991).
body. This blood has a low oxygen concentration and is therefore colored dark red. Via the right ventricle and the pulmonary artery the blood is pumped through the lungs, where oxygen enters the blood. From the lungs the now light red colored blood flows to the left atrium. The left ventricle pumps the blood into the aorta, from which it flows to all organs in the body, where e.g. oxygen leaves and carbon dioxide enters the blood. The now dark red blood flows back to the right atrium.

Figure 1.2 Cast of the coronary circulation of a goat heart. Silicone was injected into the coronary arteries. After hardening of the material, the myocardial tissue was removed. A cast of the coronary arteries that penetrate the myocardial tissue remained. Made by Aart Boekee. See cover for another example.

Like all other organs, the heart must be supplied by nutrients to maintain its function. For this supply of nutrients the heart muscle is penetrated by a network of blood vessels: the coronary circulation (Figure 1.2, cover illustration). The coronary vascular system is divided in two parts: the left and right coronary vascular system. The left and right main coronary arteries originate in the aorta, immediately behind the aortic valves. From the aorta they run along the outer surface of the heart, the epicardium, and branch into smaller arteries that penetrate the heart muscle. Arterioles, the smallest arteries, supply the very dense capillary network, in which exchange of nutrients and waste products takes place. Blood from the capillaries drains to the venules and epicardial veins, and finally to the coronary sinus, which empties into the right atrium. However, in dogs
20-30% of the blood entering the coronary circulation is drained immediately into the lumen of the right ventricle, whereas about 7% is drained into the left ventricle directly (Lendrum et al., 1945; Rayford et al., 1959; Hammond and Austen, 1967; Scharf et al., 1971; Moir et al., 1963). Although human hearts also have Thebesian drainage vessels emptying into the ventricles, no data on Thebesian flow in human hearts were found.

A primary condition for the maintenance of life is that the heart keeps beating. In humans the heart beats around 100,000 times every day and pumps 7500 liters of blood through the body. This amount of heart work is quite impressive, especially when one realizes that the 7500 liters pumped every day can fill more than 25 baths. When the heart fails, organs and tissues will become short of nutrients and will not be able to dispose their waste products. Eventually this will result in irreversible damage. For example the brain is permanently damaged after about 6 minutes without oxygen. Unfortunately, cardiovascular disease was the cause of about 40% of the human deaths in the Netherlands in 1991 (Hoogenboezem, 1993). 42% of these deaths were caused by ischemic heart disease, which results from global or local insufficient coronary blood flow. Understanding of the restricting factors for coronary flow is therefore of primary importance. To acquire this understanding, experimental research with the healthy coronary system as a reference is necessary. Animal experiments are helpful when the required interventions cannot be performed on humans. Extrapolation of data from animals experiments to human hearts should be done with care. However, it is most likely that many of the phenomena present in mammalian hearts also occur in humans.

1.2 Coronary blood flow

1.2.1 Control

Illustrations of flow regulation

Under normal circumstances, the amount of oxygen extracted during a single passage of blood through the coronary circulation of the heart is very high: about 70%. During exercise, the oxygen consumption of the heart can increase by a factor 4. Thus, the extra need for oxygen under these circumstances cannot be accommodated by an increase in oxygen extraction alone. Therefore, the coronary blood flow must be carefully adapted to the metabolic needs of the myocardium. This local control of coronary blood flow is mediated via resistance changes of the small arteries and arterioles. By adjustments of the smooth muscle tone the diameters of these vessels are adapted.

An illustration of the adjustment of flow to the needs of the myocardium
is the hypoxic dilation. Katz et al. (1955) measured a decreased coronary resistance after a decrease in coronary arterial oxygen content. Hence, coronary flow will increase after a decrease in tissue oxygen availability, and vice versa (Eckenhoff et al., 1947; Stüber et al., 1985).

A different compensatory action of the coronary circulation to a decreased oxygen supply is reactive hyperemia. Reactive hyperemia is the phenomenon that occurs after an interruption of coronary blood flow for a period of some seconds, e.g. by occlusion of the coronary artery. As a response a huge temporal increase in flow becomes apparent when the occlusion is released (Figure 1.3). This phenomenon was demonstrated early in the hearts of dogs by Katz and Lindner (1939). Since then it has been subject of many studies (e.g. Rubio et al., 1969; Bache et al., 1973; Giles and Wilcken, 1977; Olsson et al., 1978; Ruiter et al., 1978; Dole et al., 1981; Kanatsuka et al., 1992).

![Graph showing reactive hyperemic response](image)

**Figure 1.3** Reactive hyperemic response. After the release of a 15 seconds occlusion of the coronary artery, a temporal compensatory increase in coronary flow above the control level is apparent. Data are obtained from a goat with perfusion of the left main coronary artery at a constant pressure (Chapter 2).

Two well-known steady state phenomena of coronary flow control are autoregulation and metabolic flow adaptation. Autoregulation was defined by Johnson (1964) as "the intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure". Berne (1959) presented one of the first studies in which myocardial autoregulation became evident. Metabolic flow adaptation is the adaptation of flow to metabolic needs. In the literature this phenomenon is also referred to as metabolic regulation or functional hyperemia. Metabolic flow adaptation was measured first by Barcroft and Dixon (1906). Measurements by Mosher et al. (1964) illustrate both autoregulation and metabolic flow adaptation (Figure 1.4). At a control level of myocardial
performance, changes in perfusion pressure over a large range result in relatively small changes of coronary flow. At a decreased cardiac effort this autoregulatory curve is shifted parallelly.

From measurements on metabolic flow adaptation it is clear that coronary flow correlates strongly with myocardial oxygen consumption (Figure 1.4, Eckenhoff et al., 1947; Acelia et al., 1955; Mohrman and Feigl, 1978; Knabb et al., 1983). Therefore, when investigating autoregulation the oxygen consumption should be constant while varying the perfusion pressure. However, under normal circumstances the coronary perfusion pressure is equal to aortic pressure. Changes in this pressure, which are necessary to examine autoregulation, will change the cardiac afterload and thus the myocardial oxygen consumption. To be able to change the coronary arterial pressure without large changes in oxygen consumption, the coronary artery must be cannulated and perfused from a blood source independent of the aortic pressure.

The steady state relationship between coronary flow \((Q_a)\), myocardial oxygen consumption \((\text{MVO}_2)\) and coronary arterial pressure or perfusion pressure \((P_p)\) was investigated in our group by Vergroesen et al. (1987b) in the anesthetized goat with cannulated left main coronary artery. Coronary flow was affected by coronary arterial pressure and myocardial oxygen consumption according to the formula: \(Q_a = aP_p + b\text{MVO}_2 + c\). From this relationship it was concluded that a certain factor controlling coronary flow might be influenced by perfusion pressure and myocardial oxygen consumption in an independent way. The tight relation between the three variables makes it likely that autoregulation
and metabolic flow adaptation are mediated by the same control mechanism. Thus, autoregulation is in essence a demonstration of metabolic flow adaptation, namely the adaptation of flow to unchanged metabolism after changes in driving pressure.

**Mechanisms of flow control**

In the perspective of control theory, the use of the phrase 'control of flow' might be erroneous. 'Control of flow' suggests that the flow is the controlled variable. In that case, flow should be continuously monitored and compared to a preset value. In organs with flow regulation, the mechanism responsible for the flow measurement should be able to adapt the arteriolar smooth muscle tone. Such a mechanism has been found. However, as a result of increased coronary flow, both a coronary vasodilation (Lansman, 1988) and coronary vasoconstriction (Bevan and Siegel, 1991) have been reported. It should be noted that constriction is needed after a flow increase for proper control of flow. Furthermore, from the descriptions of the adaptation of flow written above it should be clear that coronary flow varies with the myocardial oxygen consumption. Thus, the setpoint of flow should depend on myocardial oxygen consumption. This analysis revealed no indications that coronary flow is the variable that is actually controlled. However, coronary flow is probably a variable that is within a control loop. The variable that actually is controlled and responsible for the flow adaptations, is still not identified. Many substances, all dependent on myocardial metabolism, have been proposed.

A lot of research has been directed to adenosine as the controlled variable in coronary flow adaptation. The production of adenosine, a potent vasodilator, is increased when the oxygen supply/demand ratio is decreased (Bardenheuer and Schrader, 1986; Sparks and Bardenheuer, 1986). The interstitial concentrations are within the range in which adenosine affects the smooth muscle cells of the resistance vessels (VanBavel, 1989). These findings support the adenosine hypothesis (Berne, 1963, 1980), that predicts that with an increased oxygen consumption the adenosine concentration will increase, which causes the resistance vessels to dilate. The control mechanism shows a negative feedback, since due to the resulting flow increase more adenosine will be washed out, causing the concentration to decrease again. However, Laird et al. (1981) modelled this hypothesis and could not predict the parallel shift of autoregulation curves at increased oxygen consumption. Furthermore, the role of adenosine as a regulator of coronary flow has become less likely due to experiments with adenosine deaminase, which was supplied to the cardiac interstitium in concentrations that are thought to break down all interstitial adenosine (Dole et al., 1985; Hanley et al., 1986; Kroll and Feigl, 1985). The adaptation of flow to the metabolic demands was not affected by the enzyme.
INTRODUCTION

The role of myocardial oxygen tension in coronary flow adaptation has been extensively studied (e.g. Dole and Nuno, 1986; Feigl, 1983 (review); Guyton et al., 1964; Vergroesen et al., 1987b, 1988). Belloni and Sparks (1977) compared the time course of changes in myocardial oxygen consumption and coronary vascular resistance after heart rate changes in dogs. The vascular transit time between the tissue and the coronary sinus was estimated by comparison of the total coronary vascular transit time, measured by dye dilution, with the arrival times of 15 \( \mu \)m radioactive microspheres in the tissue. After adjusting the time course of coronary resistance for the vascular transit effects, it was found that coronary sinus oxygen content changes preceded changes in vascular resistance. Hence, the hypothesis that coronary resistance is regulated in part by factors linked to oxidative metabolism was supported. However, the reported difference in time course of coronary sinus oxygen content and coronary resistance is likely to be overestimated, because the vascular transit time estimated for \( \text{O}_2 \) transport includes the precapillary transit time, whereas important changes in \( \text{O}_2 \) content occur distal from these vessels. The role of myocardial oxygen tension was also investigated in our group by the development of a model in which the partial oxygen pressure (\( \text{P}_{\text{O}_2} \)) of the tissue was the controlled variable in coronary flow adaptation. It was assumed that tissue \( \text{P}_{\text{O}_2} \) determined coronary resistance. Steady-state predictions of pressure-flow relations as well as dynamic responses resembled experimental observations (Drake-Holland et al., 1984; Dankelman et al., 1989b). Hence, tissue \( \text{P}_{\text{O}_2} \) may play an important role in coronary flow adaptation.

Recently Daut et al. (1990) reported that ATP-sensitive potassium channels may also be important for coronary regulation. In their experiments blocking of the channels abolished vasodilatory responses due to a coronary occlusion and anoxic perfusion. However, recent findings in our laboratory indicate that only the rate of dilatory response may have been diminished and that steady state control still may be present after blocking the channels (Dankelman et al., 1994). The regulatory responses to pressure or heart rate steps were found to be slow after blocking compared to control circumstances. Hence, although ATP-sensitive potassium channels may have little influence on steady state regulation, they are important during the dynamic adaptation.

Metabolic substances are not the only factors responsible for the coronary flow control. Transmural pressure and coronary flow through vessels also mediate the resistance of the vessels. The effect of distending pressure on vascular resistance was already discovered in 1902 by Bayliss. An increase in distending pressure resulted in a vasoconstriction, whereas a pressure decrease resulted in vasodilation. Kuo et al. (1988) discovered that this myogenic response was also present in isolated coronary arterioles (Figure 1.5) and that it was independent of endothelium (Kuo et al., 1990). Although the effect of cardiac metabolism on
this phenomenon is unclear, the myogenic response may play a role in coronary regulation. Coronary flow in itself has a dilatory effect on coronary arteries and arterioles (Holtz et al., 1983, 1984; Hintze and Vatner, 1984; Jones et al., 1993 (review)). This flow induced dilation is mediated by the endothelial cells (Kuo et al., 1991). The phenomenon causes a positive feedback since dilation of the vessel will result in a further increase in flow. Therefore this mechanism in itself cannot be responsible for the coronary flow control. However, it may be an important potentiating mechanism in coronary flow control. For example, when the smallest resistance vessels are dilated due to e.g. a metabolic signal, the resulting flow increase may cause the larger proximal vessels to dilate, so that blood supply to the tissue is increased further. It is likely, that one or more metabolic substances act together with the myogenic response and the flow dependent dilation on the coronary resistance in such a way that coronary flow is adapted to the metabolic needs of the cardiac tissue (Jones et al., 1993).

1.2.2 Effect of heart contraction

In 1695 Scaramucci already noticed that heart contraction affects myocardial flow mechanically. Since then, many studies have been directed on this phenomenon (see Hoffman and Spaan, 1990, for review). During diastole, coronary arterial flow is high while venous flow is low. During systole, the opposite is true: arterial flow is low and can even be negative in the beginning of systole, while venous flow is positive. Hence, depending on site of observation one may conclude that either heart contractions are flow impeding (arterial site) or flow augmenting (venous site). Even when flow at both sites is measured, the
effect of heart contraction is not immediately clear. It was believed that contraction stimulated flow by a pump mechanism: during relaxation blood was sucked into the myocardial vessels, and it was pumped out of it due to contraction (Wiggers, 1954). Contrarily, there was also the idea that heart contraction impeded blood flow during systole (Sabiston and Gregg, 1957). Measurements revealed that a reduction in heart rate resulted in a substantial increase in flow, either during control or maximal pharmacological vasodilation (Sabiston and Gregg, 1957; Raff et al., 1970). Hence, the latter view appeared to be correct. Heart contraction impedes myocardial perfusion. Studies on transmural flow distribution revealed that this impeding effect was most important at the subendocardium (Bache and Cobb, 1977, Downey and Kirk, 1974, 1975a; Downey et al., 1974; Hamlin et al., 1982). In maximally vasodilated hearts, disposition of microspheres injected during diastole was largest in the subendocardium, whereas during systole the subepicardial disposition was much larger. Hence, in maximally vasodilated hearts compression during systole altered the coronary resistance in such way that it reversed the diastolic flow gradient favoring the subendocardium. The mechanical flow impediment due to heart contraction obviously interacts with the flow regulation. Therefore, an increment of heart rate mediates two opposing effects on coronary flow: the increased metabolism causes flow to increase, whereas due to increased compression flow decreases. To compensate for the increased resistance to flow, the coronary arterioles must dilate.

Different models have been proposed for the mechanical interaction of heart contraction and coronary flow (Figure 1.6). The waterfall model (Downey and Kirk, 1975b) poses that during systole the increased intramyocardial pressure causes the coronary vessels to collapse at the distal site where this tissue pressure becomes higher than intravascular pressure. Driving pressure is then no longer the difference between perfusion pressure and venous pressure, but becomes smaller, namely the difference between perfusion pressure and intramyocardial pressure at the site of collapse. Intramyocardial pressure is assumed to increase transmurally from zero at the subepicardium to left ventricular pressure at the subendocardium. Thus systolic coronary flow will depend on the transmural location and will be impeded more at the subendocardium than at the subepicardium according to this hypothesis. The retrograde coronary arterial flow which is sometimes observed during systole cannot be explained by the original waterfall model. This effect could be accounted for when a small proximal compliance was added to the collapsible tube model (Lee et al., 1984; Burattini et al., 1985). However, the increased venous outflow during systole could not be explained by the waterfall model.

The intramyocardial pump model was introduced by Spaan (1981). It is based on the suggestion by Arts (1978) that the volume variations of the coronary
circulation are important in relating flow to heart contraction. Due to increased intramyocardial pressure during systole, blood is squeezed out of the compliant intramyocardial vessels. The arterial blood flow thus decreases during systole or may even be retrograde, while the venous flow increases due to this capacitive flow component. During diastole the reverse happens: the reduced compression causes vessel volume to increase. Coronary resistance depends on the volume of the coronary vessels, therefore resistive flow will vary throughout the heart cycle. The time constants for intramyocardial volume changes are large (1–2 s, Spaan et al., 1981) compared to the duration of diastole and systole. Therefore, an equilibrium is not achieved within a heart cycle.

The time varying elastance model by Krams and Westerhof (1988, 1989) is also an intramyocardial pump model. The difference with the earlier model of Spaan is the mechanism responsible for the coronary volume variations. In the earlier model the intramyocardial pressure is assumed to be the cause of the coronary volume changes. This pressure is usually assumed to be linearly related to the ventricular pressure and dependent on the depth within the ventricular wall, being equal to ventricular pressure at the endocardium and zero at the epicardium. However, in the time varying elastance model the coronary volume variations are assumed to be caused by changes in stiffness of the myocardium during the cardiac cycle. During systole, the myocardial stiffness increases, which
causes the elastance of the coronary vessels to increase. Increments of elastance cause decreases of vascular compliance. This will cause blood to leave the intramyocardial vessels and coronary volume will decrease. Thus, in contradiction with the pressure driven intramyocardial pump model, in the time varying elastance model the effect of heart contraction on flow is independent of left ventricular and intramyocardial pressure. Evidence for the elastance model is found from experiments in empty beating hearts that developed no left ventricular pressure (VanWinkle et al., 1991; Krams et al., 1989a,b,c). In these experiments, flow was still pulsatile and microspheres disposition at the subendocardium decreased in the same amount as in the normal beating heart. These findings lead to the conclusion that varying elastance is the cause of the flow impediment. However, in the in situ normal beating heart a left ventricular pressure effect on coronary flow is prominent (Kouwenhoven et al., 1992). Most likely, the two effects are interrelated in reality.

From the above it is clear that the mechanical effect of heart contraction on coronary perfusion is mediated via changes in coronary vascular volume. These volume changes determine the phasic patterns of arterial and venous flow. Coronary arteries, capillaries and veins are affected differently by the cardiac contraction. Knowledge of phasic volume variations of the different vessel types would give more insight in the mechanism causing flow impediment, however, these data cannot be measured yet. Only data on average volume per vessel type can be attained. This information can also be used to understand the time characteristics of coronary flow variations. In general, a smaller vascular volume will imply a larger coronary resistance R and a larger compliance C. Hence, the RC-time of a compartment increases with smaller volume. With increased RC-times, coronary volume changes due to contraction will be slow, and thus the coronary flow may be less pulsatile.

1.3 Survey of coronary volume measurements

1.3.1 Methods

Several methods have been developed to obtain coronary volume measurements. With some of these, only data on the total vascular volume can be obtained, whereas with others a distinction can be made between arterial, capillary and venous volumes. The different methods to measure coronary volume will be discussed below.
Indicator dilution

With this technique, an intravascular indicator is injected at the entrance of a vascular system, and the time-course of its concentration is measured at an exit of the system. A frequently used indicator is indocyanine green or CardioGreen. The mean transit time can be calculated from the time-concentration curve. From the indicator-dilution technique as originally used by Stewart (1897) and Hamilton et al. (1928) it was found that the total intravascular volume $V$ of the system can be calculated from the mean transit time and flow: $V=Q\cdot t$. Furthermore, flow appeared to equal the ratio of the amount of injected tracer and the area under the time-concentration curve. Meier and Zierler (1954) presented a theoretical basis for the indicator-dilution technique.

![Figure 1.7 Response of the concentration of an intravascular indicator in time at the exit of the coronary system. Adapted from Hirche and Lochner (1962).](image)

The tracer-dilution theory is valid in linear, constant systems. However, the coronary circulation is a non-linear system which is affected by heart contraction. Thus, coronary flow and volume are not constant. Bassingthwaighe et al. (1970) evaluated the effect of periodic variations of flow on the flow and volume estimates in a model study. The effect of variations in the system volume were not taken into account. The results indicate that the error in estimated flow and volume depends on the frequency of the flow pulsation. In the frequency range where errors were present, the error was reduced by averaging of multiple calculations that were randomized to the phase of injection. However, the error did not disappear completely: a bias dependent on the frequency of the pulsation remained. The frequency range in which errors appeared to be negligible was related to the dye curve passage time. Although a definition of this time could not be found in the article, it seems likely that the authors refer to the time period from appearance to disappearance of tracer at the system exit. Errors are
negligible when the pulsation cycle time is less than a quarter of the dye curve passage time. Obviously this time is dependent on the flow level, however, a value in the order of 15 s may be estimated for the coronary circulation from Figure 1.7. Thus, pulsations due to heart contraction, although large in amplitude, will only cause minor errors. However, the smaller pulsations due to respiration may cause substantial errors. Increment of the dye curve passage time, e.g. by injecting further away from the detection site, reduces the error due to flow pulsation caused by respiration (Scheuer-Leeser et al., 1977). However, a disadvantage of a larger indicator passage time is that recirculation of the indicator is more likely to occur. With recirculation, the tail of the indicator-dilution curve will be disturbed, and thus a large error will be made when the mean transit time is calculated. Errors induced by recirculation of the tracer can be prevented by measuring not only the concentration of the indicator leaving the vascular system, but also the time course of the indicator entering the system. Coronary volume can then be calculated by multiplication of flow with the difference in mean transit times of these curves. For proper use of the indicator-dilution theory, it is also necessary that the tracer behaves similar to blood. Also, because vascular systems often have more than one feeding artery and draining vein, assumptions have to be made on the representative behavior of the vessels where the indicator-concentration has been measured.

Morgenstern et al. (1973); Virtanen et al. (1983); Ziegler and Goresky (1971) and Hirche and Lochner (1962) used the indicator-dilution technique for the estimation of total coronary volume. In the first studies, coronary flow was measured electromagnetically, whereas in the last study flow was also calculated according to the indicator dilution theory.

**X-ray imaging of contrast**

A different approach of indicator-dilution was used by Ritman and coworkers (Hoffman and Ritman, 1987; Wang et al., 1989; Wu et al., 1992) and Canty et al. (1991), who injected an intravascular X-ray contrast indicator in the aortic root and evaluated the brightness of images obtained from a fast multislice computed tomographic (CT) scanner. Image brightness, after substraction of background signal, is assumed to be linear with contrast concentration. With this assumption, the fraction of myocardial volume that is intravascular volume can be calculated during a continuous injection of contrast material from the ratio of the brightness of a myocardial region and the brightness of the aortic root, containing blood with contrast material only, without dilution by tissue. With this method, regional coronary volume values can be obtained. However, the authors did not inject the contrast material continuously, but applied a bolus injection. The ratio of the area under the myocardial dilution curve and the area under the aortic root curve was assumed to approximate the myocardial blood volume. A
theoretical basis for this assumption is not available. An example illustrates that the volume calculation is likely to be erroneous. In the hypothetical situation with plug flow and no diffusion of indicator, two intramyocardial areas with equal vascular volumes but different flows will have different areas of the intramyocardial dilution curves, since the indicator will disappear faster from the region with high flow. Thus, by applying the described method, the volume of the region with high flow will be smaller than the volume of the region with low flow, whereas both volumes were equal. Thus, the reported regional intramyocardial volumes, which were obtained with CT-imaging after bolus injections, are probably erroneous. However, the method offers good possibilities to measure regional variations in intramyocardial volumes when continuous injections are applied. Unfortunately, such measurements have not been reported yet.

Judd et al. (1993) applied the continuous injection technique to measure global coronary volume in the isolated canine septum. The used contrast agent (ethiodol) consisted of particles whose sizes were similar to red blood cells. Red blood cells are known to be subject to the Fahraeus effect. This effect causes the red blood cells to flow faster than plasma in small vessels, thereby reducing small vessel hematocrit. This effect would cause an underestimation of vascular volume with this method. The authors tested this possibility by comparison of coronary volume values obtained with the particle contrast agent to values obtained by a radioactively labelled plasma marker (albumin, see next section). The results indicated that the maximal underestimation caused by this effect was 31%.

Radioactive plasma and/or red blood cells

The principle of dilution is also used with this method. Radioactively labelled plasma is injected into the circulation (Rakušan et al., 1969; Winbury et al., 1971; Weiss and Winbury, 1974; Miller et al., 1990). After adequate mixing in the circulation, blood samples are obtained and the heart is excised. Sometimes the heart is blotted, whereas in other experiments the heart tissue is frozen. Usually the large visible vessels are excised, and the heart is cut in pieces. Radioactivity of the heart tissue is compared to the radioactivity of the blood sample. The ratio of these values is thought to represent the blood volume of the small vessels. The same principle can be applied by using labelled red blood cells instead of labelled plasma. However, by use of either a plasma marker or labelled red blood cells, an error is introduced because the hematocrit of the small vessels is substantially less than the large-vessel hematocrit. Correction for this is possible when both labelled plasma and red blood cells are used (Gibson et al., 1946; Myers and Honig, 1964; Rakušan, 1971; Crystal et al., 1981; Eliasen et al., 1982; Gonzalez and Bassingthwaighe, 1990; Judd and Levy, 1991).
INTRODUCTION

Wüsten et al. (1977) injected radioactively labelled X-ray contrast agent in postmortem hearts. From measurements of radioactivity in transmural tissue sections, combined with coronary angiography, he determined the transmural volume gradient of small arteries. However, no comments were made on the precise penetration of contrast into the arterioles or capillaries. Therefore, it is not clear how these authors could measure arterial volume specifically. In principle, measurements of vascular volumes with radioactively labelled red blood cells or plasma do not allow distinction between the different vessel types. However, a distinction between large vessel and small vessel volume can be made. Rakušan et al. (1969) reported that after excision of the heart, freezing and removal of blood and all visible vessels, the volume of vessels smaller than 100 μm could be obtained. Without excision of the visible vessels, total blood volume could be determined. Regional volume values can be obtained with this method. A disadvantage is that intravascular pressure will most certainly decrease due to the excision of the heart. Thus, volume of the distensible coronary vessels will be underestimated.

Weight changes

Total coronary volume changes can be obtained by measuring weight changes of cannulated, isolated hearts. Pooling of blood in the atrial and ventricular spaces is prevented. This technique was used by Salisbury et al. (1961) and Scharf and Bromberger-Barnea (1973). In the former study the hearts were perfused with radioactively labelled red cells shortly before the end of an experiment. Absolute coronary volume during the measurements could then be calculated from the 'bleeding volume', i.e. the decrease in heart weight after perfusion was stopped, plus the residual radioactivity of heart muscle.

Optical hemoglobin content determination

In one of the earliest methods to obtain tissue blood content, blood and homogenized tissue were treated to convert hemoglobin and its derivates to cyanmethemoglobin. The ratio of optically determined cyanmethemoglobin concentration of undiluted tissue to that of undiluted blood represents the concentration of blood in the tissue. With this method, which was used by Klein (1945), no correction was made for myoglobin.

Histology

With this method heart tissue is fixated and examined by light or electron microscope. Usually point counting techniques are used to obtain relative cross sectional areas of different vessel types (Frank and Langer, 1974; O’Keefe et al., 1978; Weiss and Conway, 1985; Hyde and Buss, 1986; Cimini and Weiss, 1988; Judd and Levy, 1991). The relative cross sectional area is representative for the
volume of the vessel type involved. Occasionally, the diameters and density of a certain vessel type are measured and volume is calculated from these values (Gerdes et al., 1979; Gerdes and Kasten, 1980). Fixation of tissue might cause tissue shrinkage, thus introducing some uncertainty in the correctness of the volume values obtained by this method.

**Cast**

At a pressure of usually 100 mmHg\(^1\), araldite or silicone is injected into the coronary arteries. After hardening of the injected material, the tissue is removed and a cast of the vascular tree is obtained. Douglas and Greenfield (1970) occluded a large part of the coronary arterial bed with 200 μm beads before making a cast. The volume of the silicone cast was determined from the specific gravity and weight of the cast. Arts (1978) used araldite as a casting material and measured the volume of arterial vessels down to 400 μm, by determining diameters and lengths of the tree branches. They found that cast diameters were probably too large, since the diameters of the left anterior descending artery after the first side branch were similar for casts and *in vivo* hearts, whereas the weights of the *in vivo* hearts were almost twice the weight of the hearts used for the casts. Therefore, the arterial volume data obtained from the casts were corrected to obtain the *in vivo* volumes.

### 1.3.2 Results

With some of the methods described above, regional differences in volume can be measured. These data on volume heterogeneity, however, will be provided in Section 1.3.4. In this section the data on average volume measurements will be given. These results are summarized in Table 1.1. They will be discussed according to vessel type. A review of data on coronary volume has been given by Spaan (1985).

**Total coronary volume**

Using the indicator dilution technique, Morgenstern et al. (1973) detected a correlation between arterial perfusion pressure and total coronary volume in open-chest dogs with cannulated left coronary arteries. By varying coronary perfusion pressure between 70 and 170 mmHg intramyocardial blood volume increased from 11.0 to 17.8 ml/100 g left ventricle (LV). The value of 15.6 ml/100 g, as measured by Hirche and Lochner (1962) in closed chest dogs at an average aortic pressure of 131 mmHg, corresponds well with these data.

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\(^1\) For reasons of comparison, the unit of pressure used throughout this thesis is mmHg. This unit can be converted to the SI unit for pressure Pascal by multiplication with 133, hence 1 mmHg = 133 Pa.
Virtanen et al. (1983) did not find an effect of vasodilation by intravenous dipyridamole on coronary volume. However, whereas flow increased 55%, perfusion pressure fell 20% and heart rate was not constant. Absolute values of total coronary volume could not be derived from this study, since an unknown percentage of the total venous outflow was measured. In the studies mentioned above, indocyanine green was used as an indicator. Ziegler and Goresky (1971) used radioactively labelled red blood cells and plasma as indicators and estimated coronary blood volumes in vasodilated dog hearts ranging from 8 to 14 ml/100 g depending on coronary flow.

By measuring weight changes of isolated dog hearts, Salisbury et al. (1961) also found a dependency of coronary volume on arterial perfusion pressure: per mmHg pressure increase coronary volume increased 0.1 ml/100 g. Absolute values of coronary volume were calculated from residual volume, as determined by radioactivity, and bleeding volume. They ranged from 12 to 15 ml/100 g. Scharf and Bromberger-Barnea (1973) also found a dependency of coronary vascular volume on pressure by measuring weight changes of isolated dog hearts.

The coronary volumes measured with X-ray imaging after bolus injections of contrast in dogs ranged from 3-27%, depending on flow and perfusion pressure (Hoffman and Ritman, 1987; Wang et al., 1989; Canty et al., 1991; Wu et al., 1992). With continuous contrast injection, values of 7.5-12.1 ml/100 g were obtained for the maximally vasodilated, slowly beating canine septum with variations in perfusion pressure from 20 to 90 mmHg (Judd et al., 1993). The maximal underestimation of the volumes from this study due to the Fahraeus effect was 31%.

As can be seen in Table 1.1, values on total coronary volume that are obtained by microscopic examination and radioactive red blood cells or plasma are on average smaller than the values obtained by the other methods. As explained, this is probably due to the necessity of excision with these methods.

**Arterial volume**

The volume of the arteries larger than 200 μm, estimated from dog casts of the left main coronary artery, is reported by Douglas and Greenfield (1970) to be 1.3 ml/100 g total heart weight. Assuming that this artery perfused 70% of the heart, a value of 1.9 ml/100 g is obtained. Arts (1978) used a similar method and obtained for right and left coronary arteries down to 400 μm a cast volume of 1.3 ml for dog hearts with an average weight of 123 g. However, he reasoned that this value of 1.1 ml/100 g was too large compared to in vivo arterial volume. After correction he came to an in vivo arterial volume of 0.7 ml/100 g.
# Table 1.1 Survey of coronary volume data from literature

<table>
<thead>
<tr>
<th>First author</th>
<th>Animal</th>
<th>Coronary volume [ml/100 g]</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Arterial</td>
</tr>
<tr>
<td><strong>Indicator dilution</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Hirche (1962)</td>
<td>dog</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
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<td>dog</td>
<td>11.0-17.8</td>
<td></td>
</tr>
<tr>
<td>Ziegler (1971)</td>
<td>dog</td>
<td>8-14</td>
<td></td>
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<tr>
<td><strong>X-ray imaging, bolus injection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canty (1991)</td>
<td>dog</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>Wu (1992)</td>
<td>dog</td>
<td>3-27</td>
<td></td>
</tr>
<tr>
<td>Wang (1989)</td>
<td>dog</td>
<td>5-23</td>
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</tr>
<tr>
<td>Hoffman (1987)</td>
<td>dog</td>
<td>13.4/22.5</td>
<td></td>
</tr>
<tr>
<td><strong>X-ray imaging, continuous injection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Judd (1993)</td>
<td>dog</td>
<td>7.5-12.1</td>
<td></td>
</tr>
<tr>
<td><strong>Weight changes combined with radioactive RBCs</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Salisbury (1961)</td>
<td>dog</td>
<td>12-15</td>
<td></td>
</tr>
<tr>
<td><strong>Cast</strong></td>
<td></td>
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<td></td>
</tr>
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<td>Douglas (1970)</td>
<td>dog</td>
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<td></td>
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<tr>
<td>Arts (1978)</td>
<td>dog</td>
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</tr>
<tr>
<td><strong>Optical density of hemoglobin</strong></td>
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<td></td>
</tr>
<tr>
<td>Klein (1945)</td>
<td>cat</td>
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<tr>
<td><strong>Histology</strong></td>
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<td></td>
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<tr>
<td>O'Keefe (1978)</td>
<td>dog</td>
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<td>27.2</td>
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<tr>
<td>Hyde (1986)</td>
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<td>2.7</td>
</tr>
<tr>
<td>Gerdes (1980)</td>
<td>dog</td>
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</tr>
<tr>
<td>Gerdes (1979)</td>
<td>rat</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Levy (1988)</td>
<td>rat</td>
<td>0.30.7</td>
<td>2.715.9</td>
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<td>Frank (1974)</td>
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<td></td>
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<tr>
<td>Weiss (1985)</td>
<td>rabbit</td>
<td>0.6 (0.3)(^+)</td>
<td>16 (9)</td>
</tr>
<tr>
<td>Cimini (1988)</td>
<td>rabbit</td>
<td>0.4 (0.2)(^+)</td>
<td>16.6 (11.2)</td>
</tr>
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<td><strong>Radioactive plasma</strong></td>
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</tr>
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<td>Wüsten (1977)</td>
<td>dog</td>
<td>3.6(^a)</td>
<td></td>
</tr>
<tr>
<td>Weiss (1974)</td>
<td>dog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rakušan (1969)</td>
<td>dog</td>
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<td></td>
</tr>
<tr>
<td>Winbury (1971)</td>
<td>pig</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Miller (1990)</td>
<td>pig</td>
<td></td>
<td></td>
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<tr>
<td><strong>Radioactive plasma/red blood cells</strong></td>
<td></td>
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</tr>
<tr>
<td>Gibson (1946)</td>
<td>dog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myers (1964)</td>
<td>dog</td>
<td></td>
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</tr>
<tr>
<td>Eliasen (1982)</td>
<td>dog</td>
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</tr>
<tr>
<td>Crystal (1981)</td>
<td>dog</td>
<td>7.3/12.8(^a)</td>
<td></td>
</tr>
<tr>
<td>Rakušan (1971)</td>
<td>rat</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>Gonzalez (1990)</td>
<td>rabbit</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Judd (1991)</td>
<td>rat</td>
<td>5.08.6</td>
<td>0.311.2(^a)</td>
</tr>
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</table>

Legend: see next page
INTRODUCTION

Small arterial volume was measured by Wüsten et al. (1977) after injection of a radioactive barium-gelatin mixture in postmortem dog hearts. A value of 3.6 ml/100 g was reported. However, the level of vascular tone and the smallest size of the vessels that were filled with the radioactive mixture are not clear.

Values on small arterial volume obtained by microscopic examination are usually small (<0.7 ml/100 g, Table 1.1). However, during a long diastole and maximal vasodilation O'Keefe et al. (1978) measured a value of 4.9 ml/100 g for 'larger vessels', indicating the sum of arterial and venous volume. The not precisely defined 'very large vessels' were not included in this volume. Judd and Levy (1991) obtained a much smaller value for the sum of arteries and veins >100 μm in rat hearts during a long diastole: 1.2 ml/100 g. During a long systole this volume reduced to 0.3 ml/100 g.

Capillary volume

Capillary volume has been obtained in different animals from microscopic examination (Table 1.1, histology method). Values range from 5 to 17 ml/100 g, with one exceptionally high value of 27 ml/100 g (O'Keefe et al., 1978). In rabbits, Weiss and colleagues (1985; Cimini and Weiss, 1988) found that about 40 % of the capillaries are not perfused within 8 to 14 s.

From radioactive tracer studies, the volume of the capillaries cannot be measured separately. However, small vessel volume of all vessels <100 μm, including arterioles and venules, could be obtained. These data varied from 5 to 11 ml/100 g (Table 1.1). As mentioned before, the volumes acquired by both methods are probably smaller than the in vivo volumes because of the reduced transmural pressure due to excision of the heart.

Venous volume

Literature on venous volume is scarce. Hyde and Buss (1986) measured a value of 2.7 ml/100 g with a point counting technique. Levy et al. (1988) examined venous diameters and densities in histological coupes from rats and calculated venous volumes from these data. A long systole, acquired by barium contracture, resulted in a venous volume of 0.3 ml/100 g, whereas this value was 0.7 ml/100 g during a long diastole.

Legend for Table 1.1: 1 vol% is taken to be 1 ml/100 grams, Cap: capillary, SVBV: small vessel blood volume, Pp: perfusion pressure, bold: maximal vasodilation, *: radioactive X-ray contrast combined with angiography, ”: arterioles from 19-50 μm, #: from radioactive decay after bolus injection, 0: from histology
1.3.3 Volume changes

Changes of intramyocardial volume are continuously induced by the periodic contractions of the heart. From simultaneous measurements of arterial inflow and venous outflow, it is clear that coronary volume increases during diastole, whereas it decreases during systole. However, precise measurements of coronary volume changes are complicated because only a fraction of the total venous outflow can be measured in \textit{in situ} hearts. The volume change during a heart beat probably depends on heart rate and transmural pressure distribution. The heart weight tracing in the article of Salisbury et al. (1961) shows that the variation due to heart contraction is about 2 g/100 g perfused heart. Our own measurements in \textit{in situ} goat hearts with cannulated left main coronary artery and great cardiac vein indicate much smaller variations in coronary volume within heart beats: during constant arterial flow perfusion usually values of 0.2 ml/100 g were found, whereas occasionally larger values (always < 1 ml/100 g) were obtained (unpublished data).

The difference in coronary vascular volume of a rat heart in permanent systole compared to permanent diastole is much larger than the volume change per heart beat. Judd and Levy (1991), using radioactive red blood cells and plasma, measured a total systolic coronary volume of 5.0 ml/100 g, whereas during a long diastole this value had increased to 8.6 ml/100 g. By investigating the volume of the vessels >100 \( \mu \)m morphometrically during a long systole and diastole, they came to the conclusion that the major volume difference between relaxation and contraction of the muscle originated in the intramyocardial vessels smaller than 100 \( \mu \)m (Table 1.1). Salisbury et al. (1961) found an increase of 3 ml/100 g after a heart rate decrease from 150 to 60 beats/min. A long diastole caused an additional increase of 2 ml/100 g. The values found in our cannulated goat hearts perfused with constant arterial flow were again much smaller: a heart rate decrease from 130 to 60 beats/min resulted in a coronary volume increase of 0.4 ml/100 g during control and 0.8 ml/100 g during maximal vasodilation (Chapter 4). Dankelman et al. (1988) used the same method but applied smaller steps in heart rate (\( \Delta \text{HR}=30 \) beats/min). With constant flow perfusion they measured volume changes of 0.13 ml/100 g, whereas during constant pressure perfusion volume changes were larger (0.31 ml/100 g). Furthermore, with constant pressure perfusion Vergroesen et al. (1987a) measured a large coronary volume change of 3.0 ml/100 g after induction of a long diastole. The method of coronary volume change determination, integration of the difference between coronary arterial inflow and coronary venous outflow, allowed an estimation of the response time of the process necessary to achieve volume changes. A 67% volume change was realized in 1.6 s. At maximal vasodilation by adenosine a larger volume change was realized in a shorter time: the values were 4.1
ml/100 g and 1.0 s for a 67% change, respectively. A similar time constant during maximal vasodilation was measured by Kajiya et al. (1986). In this study a long diastole was induced after cessation of arterial perfusion, and the time constant of the venous outflow after reperfusion was measured.

As mentioned above, changes in perfusion pressure also induce coronary volume changes. Changes in total coronary volume of 0.07 to 0.1 ml/100 g per mmHg change in perfusion pressure were reported (Morgenstern et al., 1973; Salisbury et al., 1961; Judd et al., 1993). With X-ray imaging, Judd et al. (1993) obtained a time constant (63% change) of 3.2 s for these volume changes after sudden changes in perfusion pressure.

In conclusion, it is clear that coronary volume is dependent of perfusion pressure, flow and heart contraction. The reported response times are larger than the duration of a heart beat. This can explain the observation of smaller volume changes within a heart beat compared to the difference between long systole and long diastole.

1.3.4 Regional differences

Regional differences of coronary volume may exist because of anatomical variations of vessel diameters and densities. However, volume heterogeneity may also be caused by local variation of mechanical factors, as e.g. extravascular pressure or collagen struts (Borg et al., 1981), or metabolic factors which affect vascular tone, as e.g. myocardial oxygen consumption. With the present methods that allow regional volume measurements, a distinction between these causes for volume heterogeneity is impossible. However, the heterogenous distribution of these variables affecting coronary volume might be the cause of the coronary flow heterogeneity.

Data on transmural differences in coronary volume are inconsistent. Several studies revealed that subendocardial small vessel or capillary volume is larger than subepicardial volume (Gerdes et al., 1979; Myers and Honig, 1964; Crystal et al., 1981; Weiss and Winbury, 1974; Eliasen et al., 1982), whereas others measured no differences (Hyde and Buss, 1986; O'Keefe et al., 1978; Weiss and Conway, 1985; Cimini and Weiss, 1988) or even a larger capillary subepicardial volume (Gerdes and Kasten, 1980). Small arterial volume was reported by Wüsten et al. (1977) to be substantially larger at the subendocardium than at the subepicardium in postmortem hearts, whereas Weiss and Conway (1985) and Cimini and Weiss (1988) did not find any transmural difference in volume of arterioles with diameters from 19 to 50 μm. Large vessel volume was found to be predominantly epicardial by Judd and Levy (1991), whereas others measured a homogeneous transmural distribution (Hyde and Buss, 1986; O'Keefe, 1978). Total coronary volume was found to be homogeneously distributed by Gonzalez
and Bassingthwaigte (1990). This might be caused by averaging out the transmural differences of the different vessel types.

Differences in coronary volume between apex and base, and between left ventricle, septum and right ventricle have also been examined (Rakušan et al., 1969; Eliassen et al., 1982; Weiss and Winbury, 1974; Myers and Honig, 1964; Winbury et al., 1971; Gonzalez and Bassingthwaigte, 1990). While apical coronary volume has consistently been found to be larger than basal volume, the data on differences between left and right ventricle are again inconsistent.

1.3.5 Conclusions on volume measurements

The volume of the distensible coronary vessels is dependent of vasomotor tone and the difference between intra- and extra-vascular pressure. The contraction of the heart affects the transmural pressure difference, and may affect the compliance of the vessels directly. The conditions under which coronary volumes have been measured vary widely. Therefore, it is not surprising that data on coronary volume vary widely as well. For the interpretation of the volume measurements, it is important that the experimental conditions are precisely defined. Furthermore, to obtain realistic data, measurements should be performed under physiological conditions, i.e. in situ, pressure-developing, contracting hearts with normal perfusion pressure. Such measurements can be performed with the indicator-dilution technique.

From analysis of the time course of coronary volume changes time constants of 1-3 s have been found. These time constants are larger than the duration of a heart beat. Hence, a constant coronary volume will not be reached within a systolic or diastolic period. Due to this slow variation of coronary volume, systole and diastole cannot be regarded as independent phases of the heart cycle.

1.4 Complex interactions in the heart

1.4.1 Gregg’s phenomenon

The oxygen consumption of the heart is related to the mechanical work the heart has to deliver. E.g. heart rate and pressure in the aorta against which the heart has to pump, the afterload, are important determinants of myocardial oxygen consumption. Besides the effect of afterload on the mechanical work of the heart, myocardial oxygen consumption is also affected by the afterload in another way. The aortic pressure is also the perfusion pressure at the entrance of the coronary circulation. In 1963 Gregg reported that an increase in this perfusion
pressure resulted in itself in an increase in myocardial oxygen consumption. Measurement of this phenomenon was possible since the left coronary artery was perfused with blood from a constant pressure reservoir, and therefore perfusion pressure could be changed without a change in mechanical work induced by an afterload change. Since the first observation of 'Gregg's phenomenon', many studies on this effect were performed (see Feigl, 1983; for review; Miller et al., 1987; Kitakaze and Marban, 1989; Goto et al., 1991; Schouten et al., 1992). Changes of perfusion pressure as well as coronary flow were reported to induce myocardial \( O_2 \) consumption changes and changes in contractility. Some recent studies, however, report absence of a Gregg effect in pigs (Miller et al., 1990; Schulz et al., 1991; Schwartz et al., 1992).

Three different hypotheses have been presented to explain Gregg's phenomenon: improved perfusion, garden hose effect and increased contractility. These hypotheses will be explained in the introduction of Chapter 4.

1.4.2 Scientific context of the thesis

The coronary circulation is complex because many factors determine its behavior. These factors include tree geometry, vascular compliance, regulation and mechanical effects of heart contraction. In a solid, passive system the relation between flow and pressure is simple: flow is equal to the driving pressure divided by the resistance of the system. The resistance depends on the dimensions of the system. In general, when tube length is constant, resistance will be smaller when volume is larger. A vascular bed might be represented by many tubes with varying diameters and lengths. In such a system, the relation between volume and resistance is already complex. The compliance of the vessels complicates matters further, since vascular volume will change when distending pressure varies. This volume change will result in resistance changes. As a consequence, the flow and the intravascular pressure distribution will alter, and thus passive resistance is affected again. Incorporation of the active adaptation of resistance of the arterial part of the system to metabolic needs also affects the pressure and resistance distribution. Thus, pressure, flow and oxygen consumption all interact, and the values of these variables at each place in the vessel system depend on the values at all other places in the system. This reasoning holds for all organs. However, the continuously varying mechanical forces on the coronary vascular system, which are caused by the heart contraction, are special for the heart. Obviously, these forces also affects pressure and resistance distribution. Furthermore, in the previous section it was described that coronary perfusion pressure and flow affect myocardial oxygen consumption without directly changing parameters that influence mechanical work. This Gregg’s phenomenon might also be mediated by coronary volume changes.
In conclusion, the vascular tree characteristics combined with effects of coronary regulation and heart contraction determine the coronary perfusion at each spot in the myocardium. Measurement of these factors and their interaction at the local level would provide insight in the mechanisms that determine coronary perfusion. This understanding is important, because insufficient local perfusion will cause damage to the myocardial tissue that may eventually result in infarction. However, at this moment it is impossible to obtain these local measurements. In this thesis, some of the interactions as outlined above were evaluated at the global level. The dynamic oxygen extraction curve could be used to obtain coronary volume estimations in \textit{in situ}, contracting hearts with normal perfusion pressure. These data may be helpful for the elucidation of the mechanism by which heart contraction affects coronary perfusion. E.g. to obtain a rough estimate of the vascular compliance of the different vessel types, the volume of a coronary compartment can be multiplied by the distensibility of a certain vessel type. Additionally, the direct effect of heart rate on coronary volume may give more direct information on this matter. Furthermore, the static and dynamic oxygen extraction measurements made it possible to study the interaction between pressure, heart rate and oxygen consumption at the global level.

1.5 Contents of the thesis

In this thesis, interactions between coronary perfusion, heart contraction and oxygen consumption are studied at the global level.

In Chapter 2 a method is described that allows an estimation of a coronary volume distribution in the \textit{in situ} heart. With the use of a model of the coronary circulation, the response of arterio-venous oxygen content difference (AVo$_2$) after a step change in coronary flow is simulated and fitted to the measured response. With this method, the volume of the vessels participating in oxygen exchange and the venous volume can be obtained. Thus, a volume distribution according to vessel function is made. The results depend on the model used to represent the coronary circulation. The effect of model choice on the volume estimates is evaluated in Chapter 3. Furthermore, the method is based on the mass balance of oxygen. Therefore, the myocardial oxygen consumption time course should be known. In steady state, oxygen consumption can be calculated by multiplication of coronary flow and AVo$_2$. The applied flow step caused oxygen consumption to change in the same direction, this is an expression of 'Gregg's phenomenon'. As described in Chapter 4, heart rate was found to affect Gregg's phenomenon. Although steady state oxygen consumption can be calculated quite easily, the time course after a flow step cannot be measured or calculated directly.
Therefore, an assumed time course was used to estimate the coronary volume distribution. However, when the coronary volumes are known, the same coronary circulation model can be used to estimate the oxygen consumption time course. This approach was used in Chapter 5 to investigate the dynamics of oxygen consumption. The oxygen consumption time course after abrupt changes in perfusion pressure during control was compared to the time course after infusion of glibenclamide, a blocker of ATP-sensitive potassium channels. Blockade of these channels decreases the rate of regulation in the coronary circulation. Myocardial oxygen consumption showed a rapid change in the direction of the perfusion change, followed by a slower return to almost its initial value. After glibenclamide administration the $O_2$ consumption transients are less rapid than during control. It can be reasoned that capillary pressure changes in a similar way. Hence, the $O_2$ consumption transients are probably a demonstration of Gregg's phenomenon. In Chapter 6, the effect of heart rate on coronary volume was estimated using the method described in Chapter 2. During maximal vasodilation, the estimations increased with heart rate, whereas a volume decrease was measured by integrating the difference of arterial and scaled venous flow after a heart rate increase. The discrepancy between these results was investigated. Finally, in Chapter 7 conclusions are drawn.
Functional distribution of coronary vascular volume in the beating goat heart

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Using hemoglobin bound O₂ as an endogenous tracer, intramyocardial blood volume distribution between vessels involved in oxygen exchange and more distal vessels was estimated. In nine anesthetized open chest goats the left main coronary artery was cannulated and perfused at constant flow. Coronary arterio-venous oxygen content difference, AVO₂, was measured. AVO₂ transients induced by a flow step could be characterized by two phases: 1. delay time, 2. slow change to a new steady state. AVO₂ responses were fitted by a two compartmental model consisting of a well-mixed compartment from which oxygen is consumed with volume $V_m$ and a distal unmixed compartment without O₂ exchange with volume $V_{umn}$. The rate of change of the AVO₂ transient depends on $V_m$, while the delay time depends on $V_{umn}$.

Measurements in nine goats resulted in $V_m = 9.9 \pm 1.1$ ml/100 g (mean±SE, n=9) and $V_{umn} = 3.8 \pm 0.3$ ml/100 g. Maximal vasodilation caused a significant increase in $V_m$ (13.1±1.3 ml/100 g, n=5), while $V_{umn}$ did not change (5.0±0.5 ml/100 g). Hence, the increase of intramyocardial blood volume induced by vasodilation must be looked for in the capillary bed and not in the coronary veins.

### 2.1 Introduction

Understanding coronary mechanics requires realistic estimates of volume distribution over different vascular compartments under normal operating conditions, i.e. normal blood flow, pressure and heart contraction (Bruinsma et al., 1988, Downey and Kirk, 1975b, Krams et al., 1989b). Similarly, interpretation of the dynamics of coronary blood flow and tissue oxygen pressure control requires knowledge about the volume of the vascular compartment involved in oxygen exchange (Dankelman et al., 1989a). Although total coronary blood volume has been estimated under physiological conditions, by applying methods such as dye dilution (Hirche and Lochner, 1962, Morgenstern et al., 1973), heart weight changes (Salisbury et al., 1961) and X-ray contrast combined with fast CT-scan (Canty et al., 1991, Wu et al., 1992), data on volume distribution are lacking. Data on volume distribution has hitherto been obtained from hearts which were first excised, sometimes after these had been frozen.
In this study a functional distribution of coronary volume was estimated. To do this, the response of the arterio-venous oxygen content difference (AVO₂) of the beating heart after a step in coronary arterial flow was measured during open thorax experiments. This signal was interpreted by a model for the coronary circulation consisting of two compartments: 1. the vessels participating in oxygen exchange were assumed to be well mixed, 2. the vessels distal of these were assumed to be unmixed. Through comparison of simulations from this model and measured signals, the volumes of the two compartments can be estimated. In this way hemoglobin bound oxygen is used as an endogenous tracer for the estimation of a functional volume distribution of the coronary circulation under physiological conditions.

2.2 Methods

2.2.1 Preparation

Nine goats weighing 18 to 28 kg were used in the present study. The goats were treated for worms using ivermectine (MSD) two weeks prior to the experiment. The goats were anesthetized by intramuscular injection of a mixture of 3 ml ketamine-hydrochloride (Aescoket, 100 mg/ml), 3 ml Rompun (20 mg/ml) and 4 ml atropine-sulphate (0.5 mg/ml). Anesthesia was maintained by intravenous injection of 30 ml fentanyl (0.05 mg/ml). 2 ml pancuroniumbromide (Pavulon, Organon, 2 mg/ml) was given intravenously for muscle relaxation. The goats were ventilated with a Harvard respirator using a 2:1 nitrous oxide-oxygen mixture.

A left thoracotomy was performed and the 3rd and 4th ribs were removed. The pericardium was opened and a cradle was formed. The left main coronary artery was dissected and a ligature was placed around it. Another ligature was placed around the great cardiac vein, close to the junction where the left hemiazygous vein drains into the coronary sinus. The His bundle was destroyed by local injection of formaldehyde (37 %) and the right ventricle was paced at a constant heart rate of 90 beats per minute. A thin catheter was inserted into the left anterior descending vein according to the Herd-Barger technique (Herd and Barger, 1964). A catheter tip manometer (PPG Hellige, model HD 36 010 80001, 6F) was inserted through a purse string in the left atrial appendage into the left ventricle. A stainless-steel Gregg cannula was inserted into the aorta via a purse string after administration of 3 ml heparin (5000 IU/ml iv). Without disruption of coronary flow, the cannula was ligated into the left main coronary artery. The great cardiac vein was cannulated via the left hemiazygous vein. Anticoagulation was maintained by continuous infusion of heparin (5000 IU/hour).
2.2.2 Arterial perfusion/venous drainage system

The arterial perfusion system and venous drainage system are drawn in Figure 2.1. The arterial perfusion system was essentially similar to the one previously described by Spaan et al. (1981). Blood from the left carotid artery was pumped into a 70 ml reservoir, approximately half filled with blood, via a heat exchanger and filter (Mirospore, 40 μm). A precision pressure regulator (Fairchild, model 10R) connected to a laboratory compressed air system held the reservoir pressure at a preset value. Perfusion pressure was measured at the cannula tip with a catheter tip manometer (Millar SPC-350, 5F). An electromagnetic cannulating flow probe, interposed in the perfusion line, monitored coronary arterial flow (Statham, inner diameter 5 mm, model Sp2202). The blood reservoir was equipped with a level controller (M.S.A. automatic suction control) with feedback to the roller pump, in order to keep the blood level in the reservoir constant.

Blood from the cannulated great cardiac vein drained into a similar pressure controlled reservoir via a 2.5 mm flow probe (Statham, model Sp2202).

![Diagram of Arterial Perfusion and Venous Drainage System](image)

**Figure 2.1** Arterial perfusion and venous drainage system. See text for details. LM: left main coronary artery, GCV: great cardiac vein, CA: carotid artery, JV: jugular vein, EM: electromagnetic flow probe, AV: cuvette for measurement of the arterio-venous oxygen content difference.
The pressure in the reservoir was regulated at a preset value. Venous blood was pumped back into the left jugular vein via a heat exchanger and filter (Miropore, 40 μm).

For dynamic measurement of the arterio-venous $O_2$ content difference, two cuvettes were used. The arterial cuvette was placed in the arterial perfusion system before the pressure controller. The venous cuvette was placed directly after the venous cannula.

2.2.3 Dynamic measurement of the arterio-venous $O_2$ content difference

The arterio-venous $O_2$ content difference was measured dynamically with a device first described by Shepherd et al. (1975), which was adapted to obtain faster responses as reported by Vergroesen and Spaan (1988). We slightly modified the cuvettes to make them more robust. The technique is based on measurement of the color difference at 660 nm between arterial and venous blood caused by the difference in hemoglobin $O_2$ saturation.

2.2.4 Protocol and measurements

Left ventricular, coronary arterial and venous pressures as well as coronary arterial and venous flow and arterio-venous $O_2$ content difference were continuously recorded on a Hewlett-Packard F.M. instrumentation recorder (HP 3968A) for analog backup. A/D conversion was done on-line at a sample rate of 80 Hz for at least 60 s, starting 15 s before the flow step. Digitized data were stored on hard disk.

Each protocol started with a 15 s occlusion of the arterial perfusion line and a 2 s occlusion of the venous drainage line to check for the zeros of the flow probes. This was repeated several times during each protocol. Between the occlusions, the perfusion condition was changed from constant pressure perfusion to constant flow perfusion by placing a resistance on the perfusion line distal to the pressure reservoir and raising the pressure in the reservoir to compensate for the induced resistance. Flow steps were induced by stepwise changing the reservoir pressure between two levels. After 4 steps between two preset flow levels, two different flow levels were established, and the interventions were repeated. In each experiment, at least two groups of 4 flow steps were performed. In five goats, the protocol was repeated at vasodilation with adenosine. To test for maximal dilation, the arterial perfusion line was clamped for 15 s during constant pressure perfusion. If reactive hyperemia had not yet disappeared completely, the intracoronary infusion rate of adenosine (5 mM) was increased. The flow steps were repeated at flow controlled perfusion.

Measurements without stable arterio-venous $O_2$ content differences before
or after the flow step were excluded from the data analysis.

The pH, arterial Po$_2$ and Pco$_2$ were measured every 30 minutes with an automated blood gas analyzer (model ABL330, Radiometer). Hemoglobin content, arterial and coronary venous oxygen saturations were measured with a hemoximeter (model OSM 2, Radiometer) before each intervention. These data were used for calibration of the dynamic arterio-venous O$_2$ content difference measurement.

The heart was excised at the end of the experiment. A mixture of gelatine and white paint (Latex) at 37°C was injected into the left main coronary artery. After cooling in a freezer for approximately half an hour, the colored tissue could be dissected and weighed.

2.2.5 Estimation of the coronary volume participating in O$_2$ exchange and the distal coronary venous volume

Model

For estimation of the coronary volume distribution, the measured time response of the arterio-venous O$_2$ content difference, AVo$_2$(t), to a flow step was compared with model predictions. The model is a simplified representation of the coronary vascular bed and consists of a well-mixed compartment from which O$_2$ is consumed, and a compartment without O$_2$ exchange (Figure 2.2). The volumes of the two compartments, which are assumed not to be affected by the intervention, are the parameters to be estimated.

Because of the oxygen mass balance, the difference between in- and outflow of oxygen must equal the sum of oxygen consumption and change in stored oxygen. For the mixed compartment, this can be written as follows:

$$\frac{dA(t)}{dt} = Q_a(t) \cdot [O_2]_a(t) - Q_a(t) \cdot [O_2]_m(t) - MVo_2(t)$$  \hspace{1cm} (2.1)

where:

- \( t \) = time
- \( A(t) \) = total amount of O$_2$ in blood and tissue in the mixed compartment [\( \mu l \) O$_2$/100 g]
- \( Q_a(t) \) = arterial flow [ml/s/100 g]
- \([O_2]_a(t)\) = arterial O$_2$ content [\( \mu l \) O$_2$/ml blood]
- \([O_2]_m(t)\) = O$_2$ content of blood in the mixed compartment [\( \mu l \) O$_2$/ml blood]
- MVo$_2(t)$ = myocardial O$_2$ consumption [\( \mu l \) O$_2$/s/100 g]

Oxygen consumption is written as a function of time since it may change during the response to the flow step as a result of the Gregg effect (Gregg, 1963).
Figure 2.2 The model of the coronary vascular bed used to simulate the time course of the arterio-venous O$_2$ content difference consists of two compartments: a well-mixed compartment from which O$_2$ is exchanged, and an unmixed compartment without O$_2$ exchange. The volumes of these compartments, $V_m$ and $V_{unm}$, were assumed to be constant during an intervention. $Q_a$: coronary arterial flow, $[O_2]_a$: arterial oxygen concentration, $[O_2]_m$: oxygen concentration in the mixed compartment, $MV_{O_2}$: myocardial oxygen consumption.

The amount of oxygen in this compartment at time t, A(t), is the sum of O$_2$ in blood and in tissue:

$$A(t)=V_m[O_2]_m(t)+V_t[O_2]_t(t)$$  \hspace{1cm} \{2.2\}

where:

- $V_m = \text{volume of blood in the mixed compartment} \ [\text{ml/100 g}]$
- $V_t = \text{volume of tissue in the mixed compartment} \ [\text{ml/100 g}]$
- $[O_2]_t(t) = \text{O}_2 \text{ content in tissue} \ [\mu l \text{ O}_2/\text{ml tissue}]$

The oxygen content in blood depends on the oxygen binding capacity of hemoglobin, whereas in tissue the oxygen binding capacity of myoglobin and oxygen solubility should be accounted for:

$$A(t)=V_m \cdot S_{Hb}(t) \cdot 1.36 \cdot 10^{-6} \cdot \text{Hb}+V_t(S_{Mb}(t) \cdot 1.36 \cdot 10^{-6} \cdot \text{Mb}+\sigma_t \cdot \text{Po}_2(t))$$  \hspace{1cm} \{2.3\}
where:

\[ S_{Hb}(t) = \text{hemoglobin saturation [-]} \]
\[ S_{Mb}(t) = \text{myoglobin saturation [-]} \]
\[ 1.36 = O_2 \text{ binding capacity of hemoglobin or myoglobin [ml O}_2\text{/g]} \]
\[ \text{(Schmidt and Thews, 1989)} \]
\[ Hb = \text{hemoglobin content [g/100 ml blood]} \]
\[ Mb = \text{myoglobin content [g/100 ml tissue]} \]
\[ \sigma_t = O_2 \text{ solubility of extravascular tissue [\mu l O}_2\text{/ml tissue/mmHg]} \]
\[ Po_2(t) = \text{partial O}_2 \text{ pressure [mmHg]} \]
\[ 10 = \text{factor to arrive at the dimension \mu l O}_2\text{/ml} \]

Because the volumes are assumed to be constant during one intervention, the change in the amount of oxygen in the mixed compartment can be written as:

\[
\frac{dA(t)}{dt} = V_m \frac{d(S_{Hb}(t)\cdot1.36\cdot10\cdot Hb)}{dt} + V_t \frac{d(S_{Mb}(t)\cdot1.36\cdot10\cdot Mb + \sigma_t \cdot Po_2(t))}{dt}
\]

Equation 2.4

Oxygen content of blood and tissue are related. This relation may be estimated assuming that oxygen pressure is equal in blood and tissue. The changes in saturation are related to changes in oxygen pressure according to the respective saturation curves. These curves are non-linear but may be linearized around a working point defined by the average tissue \( Po_2 \). Because of the assumption of a well-mixed compartment, this is the venous oxygen pressure. Equation 2.4 can be rewritten such that the dependent variable equals the measured variable, i.e. venous oxygen content \([O_2]_m(t)\). Hence, the variation in oxygen saturation changes and changes in dissolved oxygen are expressed in terms of a variation of venous oxygen content. Taking into account Equation 2.4, this results in:

\[
\frac{dA(t)}{dt} = (V_m + V_t \frac{\lambda_{Mb} \cdot 1.36 \cdot 10 \cdot Mb + \sigma_t}{\lambda_{Hb} \cdot 1.36 \cdot 10 \cdot Hb}) \cdot \frac{d[O_2]_m(t)}{dt}
\]

where:

\[ \lambda_{Hb} = \text{slope of linearized } Po_2 \text{ hemoglobin saturation curve [mmHg}^{-1}] \]
\[ \lambda_{Mb} = \text{slope of linearized } Po_2 \text{ myoglobin saturation curve [mmHg}^{-1}] \]

It should first be appreciated, based on estimates of parameters from literature, that the second term between brackets only amounts to 15% of \( V_m \). Hence, high accuracy of individual parameters in this term is not required. For our estimation procedure \( V_t \) was assumed to be constant and equal to 80% of
heart volume. Hb was measured from arterial blood samples. $\lambda_{Hb}$ followed from published saturation curves of goat blood (Altman and Dittmer, 1970). The saturation curve for goat myoglobin as well as goat myoglobin concentration could not be found in literature. We used human values: a myoglobin concentration of 0.4 g/100 ml tissue (Schmidt and Thews, 1989) and the human myoglobin saturation curve as described by Hill with a Hill parameter of 1 (Popel, 1989). Oxygen solubility of extravascular tissue was assumed to be 0.028 $\mu$l O$_2$/ml tissue/mmHg (Altman and Dittmer, 1970).

Combining Equations 2.1 and 2.5 yields the following mass balance equation for the mixed compartment:

$$(V_m + C) \cdot \frac{d[O_2]_m(t)}{dt} = Q_a(t) \cdot [O_2]_a(t) - Q_a(t) \cdot [O_2]_m(t) - MV_{O_2}(t) \quad \{2.6\}$$

where the correction term C in ml/100 g for the change in tissue O$_2$ storage is:

$$C = V_t \cdot \frac{\lambda_{Mb} \cdot 1.36 \cdot 10 \cdot Mb + \sigma_t}{\lambda_{Hb} \cdot 1.36 \cdot 10 \cdot Hb} \quad \{2.7\}$$

When O$_2$ consumption is constant, Equation 2.6 is a first order differential equation. In this case, the time response of [O$_2$]$_m$ to a step in flow will be exponential and can be characterized by a time constant $\tau = (V_m + C)/Q_{a,2}$, where $Q_{a,2}$ is the arterial flow after the flow step.

The second compartment only causes a time delay ($\tau_d$) in appearance of changes of the venous O$_2$ content dependent on its volume ($V_{unn}$) and flow:

$$\tau_d = \frac{V_{unn}}{Q_{a,2}} \quad \{2.8\}$$

[O$_2$]$_a$ is practically constant. Hence, $AV_{O_2}(t)$ and [O$_2$]$_m$ are related:

$$AV_{O_2}(t) = [O_2]_a - [O_2]_m(t-\tau_d) \quad \{2.9\}$$

Combining Equations 2.6 and 2.9 yields:

$$-(V_m + C) \cdot \frac{dAV_{O_2}(t + \tau_d)}{dt} = Q_a(t) \cdot AV_{O_2}(t + \tau_d) - MV_{O_2}(t) \quad \{2.10\}$$
Equations 2.8 and 2.10 together provide the mass balance of the two compartmental model. The parameters $V_m$ and $V_{unn}$ can be estimated by fitting the solution of the equations to measured responses of $\Delta V_{O_2}(t)$. However, such a procedure requires knowledge of $M\Delta V_{O_2}(t)$.

$O_2$ consumption

To estimate volumes from the time response of $\Delta V_{O_2}(t)$ to a flow step, the time course of $O_2$ consumption must be known (see Equation 2.10). Steady state values of $O_2$ consumption before and after the flow step could be calculated from the measured flow and arterio-venous $O_2$ content difference:

$$M\Delta V_{O_2} = Q_a \cdot \Delta V_{O_2}$$  \hspace{1cm} (2.11)

As could be expected from the findings of Gregg (1963), in most interventions steady state $O_2$ consumption changed with the flow step in the same direction as the flow step. The time course of this $O_2$ consumption change is unknown. In our calculations, we assumed an exponential time course, starting at the moment of the flow step. Vergroesen and Spaan (1988) measured a 50% change in $O_2$ consumption due to cardiac arrest in open chest goats in 3.8 s. Assuming an exponential time course, this corresponds to a time constant of 5.5 s. Van Beek and Westerhof (1991) measured the mean response time of venous $O_2$ content after heart rate changes in Tyrode-perfused isolated rabbit hearts. They deduced from their data a mean response time of cardiac mitochondrial $O_2$ consumption of 7.7 s. In our model predictions, we used a time constant of 6 s, which was between these findings. However, to assess the sensitivity of the volume estimations on the assumed time course of $O_2$ consumption, calculations were repeated with time constants of 3 and 9 s.

Fitting procedure

Since the model equations do not account for phasic flow, the measured signals were averaged per beat and assumed to be discrete measures of the continuous signals. A heart beat was defined as the period between the onset of two subsequent systoles.

Model simulations were fitted to the measured arterio-venous $O_2$ content difference by varying time delay and mixed volume and using a least squares method. Measured arterial flow and an $O_2$ consumption time course as described before were used as input to the model. The moments at which the measured $\Delta V_{O_2}(t)$ had changed relatively 10 and 90% determined the time period for which the sum of squared differences between measured $\Delta V_{O_2}(t)$ and model simulations was minimized.

The sum of least squares was calculated for discrete values of mixed
volume and time delay: it was calculated for all combinations of 20 delay times ranging from 0 to 19 heart beats and 40 mixed volumes \((V_{m}+C)\) varying between 1 and 40 ml/100 grams. Hence, a matrix of 20x40 was obtained expressing the sum of least squares as discrete function of mixed volume and delay time. The minimum of this matrix revealed the uncorrected mixed volume and time delay resulting in the best fit of the model.

The unmixed volume followed from the product of arterial flow and corrected time delay. Time delay was corrected for the transport time of blood through the venous cannula. This results in:

\[
V_{\text{unm}} = Q_a \cdot (\tau_{d,\text{total}} - \tau_{d,\text{can}}) = Q_a \cdot (N \cdot \Delta t - V_{\text{can}}/Q_v) \quad \{2.12\}
\]

where:
- \(\tau_{d,\text{total}}\) = total time delay [s]
- \(\tau_{d,\text{can}}\) = time delay caused by venous cannula [s]
- \(N\) = number of heart beats shifted
- \(\Delta t\) = duration of a heart beat [s]
- \(V_{\text{can}}\) = volume venous cannula = 1.3 ml
- \(Q_v\) = venous flow [ml/s]

In simulations, four times smaller steps in mixed volume or time delay did not improve the mean and standard error of the estimations. The noise on the arterio-venous \(O_2\) content difference appeared to be dominant.

2.2.6 Estimation of change of total coronary volume induced by the flow step

The change of total coronary vascular volume induced by flow changes was calculated from the integrated difference between arterial inflow and venous outflow: \(\Delta V(t) - \int_0^t Q_a(\tau) - \alpha Q_v(\tau) d\tau\) as described by Vergroesen et al. (1987a). The flow from the great cardiac vein is only a fraction of the total venous outflow. Therefore \(Q_v\) had to be corrected by a factor. This factor \(\alpha\), being the ratio of \(Q_a\) and \(Q_v\) in steady state, causes \(\Delta V(t)\) to be zero in a period of steady state.

Two phenomena disturbed the volume change calculation. First, due to ventilation \(\Delta V(t)\) variated periodically. Second, \(\alpha\) often changed slightly due to the flow step, resulting in different steady state values before and after the flow step. To minimize the effect of ventilation on the determination of volume change, \(\Delta V(t)\) was calculated over a full number of periods. In order to assess the influence of the change of \(\alpha\), the volume change of the coronary bed was calculated using two methods: with a constant \(\alpha\), equal to the \(Q_a/Q_v\) ratio before
Table 2.1 Hemodynamic data and heart weight during control and maximal vasodilation.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maximal vasodilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean $P_L$ [mmHg]</td>
<td>31 ± 3</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>$P_p$ [mmHg]</td>
<td>102 ± 4</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>$P_{epic.ven}$ [mmHg]</td>
<td>9.9 ± 1.5 (n=8)</td>
<td>7.9 ± 0.9 (n=4)</td>
</tr>
<tr>
<td>$Q_a$ [ml/s/100 g]</td>
<td>1.4 ± 0.1</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>$Q_e$ [ml/s/100 g]</td>
<td>0.7 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Heart rate [beats/min]</td>
<td>90 ± 1</td>
<td>91 ± 1</td>
</tr>
<tr>
<td>MVO$_2$ [µl O$_2$/s/100 g]</td>
<td>79 ± 5</td>
<td>67 ± 7</td>
</tr>
</tbody>
</table>

Heart weight [g]:
- total                  | 131 ± 9       |
- left ventricle         | 76 ± 5        |
- perfused area           | 78 ± 5        |

Means ± SE, n = 9 goats for control, n = 5 goats with maximal vasodilation. In one goat epicardial venous pressure could not be measured. $P_L$: left ventricular pressure, $P_p$: perfusion pressure, $P_{epic.ven}$: epicardial venous pressure, $Q_a$: coronary arterial flow, $Q_e$: coronary venous flow, MVO$_2$: myocardial oxygen consumption.

the flow step, and with $\alpha$ changing stepwise from the value in steady state prior to the steady state value after the flow step.

2.3 Results

Average pressures, flows, heart rate and oxygen consumption during the interventions used in the analysis are provided in Table 2.1, grouped according to control and vasodilation. After maximal vasodilation with adenosine, flow was increased and perfusion pressure was decreased compared with control. On average the flow step was about 19% of total flow and the resulting change in O$_2$ consumption was 6%.

A typical time response of the arterio-venous O$_2$ content difference to a step change in arterial flow is shown in Figure 2.3. At $t=15$ s the arterial flow was changed stepwise. The response of the AVO$_2$ is characterized by 2 stages: 1. a time delay and 2. a slow change to a new steady state value. Left ventricular pressure remained constant, while the perfusion pressure increased due to the increased flow and later as a result of the increased vascular resistance due to autoregulation. Except for the direction of the changes, the responses to flow decrements were similar to the responses to flow increments.

In Figure 2.4, the arterial flow and the arterio-venous O$_2$ content difference from Figure 2.3 are averaged per heart beat. In the figure the model simulation that fitted best to the measured signal is also shown.

Table 2.2 provides the estimated volumes using a time constant of 6 s for
change in O₂ consumption. The reported mixed volumes were corrected for the influence of changes in tissue oxygen stores. This correction term varied between 0.8 and 3.0 ml/100 grams depending on the hemoglobin content and working point for the linearization of the Po₂ saturation curves. After correction and averaging the results of 9 goats, blood volumes were 9.9 ± 1.1 ml/100 g perfused tissue for the mixed compartment and 3.8 ± 0.3 ml/100 g for the unmixed compartment (means±SE). In 5 goats adenosine was administered to cause maximal vasodilation. A paired student t-test indicated that administration of adenosine significantly increased the mixed volume (P<0.02), whereas the unmixed volume did not change significantly (P>0.2). The average corrected values at maximal vasodilation were 13.1 ± 1.3 ml/100 g for the mixed volume and 5.0 ± 0.5 ml/100 g for the unmixed volume. The direction of the flow steps
Figure 2.4 Signals averaged per heart beat. Top: Coronary arterial flow $Q_a$. Bottom: Measured arterio-venous oxygen content difference ($AVO_2$, solid line) and model simulation that fitted best (broken line).

did not affect the volume estimates.

The results of Table 2.2 were obtained assuming a time constant for oxygen consumption change of 6 s. Use of different time constants (3 and 9 s) altered the volume estimations: whereas the effect on the unmixed volume estimation was small (on average less than 4%), the effect on the mixed volume was more significant. On applying time constants of 3, 6 and 9 s, the respective averaged mixed volumes were 8.5, 9.9 and 10.6 ml/100 g during control and 11.9, 13.1 and 13.8 ml/100 g during maximal vasodilation.

The flow steps caused changes in the total coronary volume in all animals. Calculations of the volume changes with a constant factor $\alpha (=2)$ revealed slightly higher values than calculations in which $\alpha$ was changed stepwise. Since the actual volume change probably is a value intermediate of the two estimates, the change of $\alpha$ due to the flow step did not seriously affect the determination of the average total volume change. With $\alpha$ constant, we estimated a volume change of 0.51 ± 0.05 ml/100 g (mean of 9 goats ± SE), while after maximal vasodilation this value was increased to 0.83 ± 0.16 ml/100 g (mean of 5 goats ± SE). Although the total coronary volume changed significantly due to the flow step, this change was small compared with the sum of the mixed and unmixed volumes (3.7% and 4.5% at control and maximal vasodilation, respectively).
Table 2.2 Results of volume estimations in ml/100 g.

<table>
<thead>
<tr>
<th>Goat</th>
<th>$n_i$</th>
<th>$V_{m} \pm SE$</th>
<th>$V_{unn} \pm SE$</th>
<th>$n_i$</th>
<th>$V_{m} \pm SE$</th>
<th>$V_{unn} \pm SE$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>9.6 ± 0.6</td>
<td>2.2 ± 0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>6.4 ± 0.5</td>
<td>3.2 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>10.9 ± 1.4</td>
<td>5.6 ± 1.0</td>
<td>5</td>
<td>17.0 ± 2.8</td>
<td>4.0 ± 2.2</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>14.0 ± 0.9</td>
<td>3.7 ± 0.2</td>
<td>8</td>
<td>15.6 ± 1.9</td>
<td>6.9 ± 0.8</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>11.3 ± 3.9</td>
<td>4.7 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>15.1 ± 1.1</td>
<td>4.4 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>6.1 ± 0.4</td>
<td>3.2 ± 0.2</td>
<td>12</td>
<td>11.8 ± 1.1</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>6.7 ± 0.3</td>
<td>4.2 ± 0.1</td>
<td>8</td>
<td>10.3 ± 0.8</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>8.7 ± 0.6</td>
<td>3.2 ± 0.4</td>
<td>12</td>
<td>10.6 ± 1.3</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9.9 ± 1.1</td>
<td>3.8 ± 0.3</td>
<td>13.1</td>
<td>5.0 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Means per goat ± SE, $n_i$ = number of interventions. $V_{m}$: vessel volume of mixed compartment. $V_{unn}$: volume of unmixed compartment distal of mixed compartment. Bottom: averaged results ± SE from 9 goats without adenosine and from 5 goats with maximal vasodilation.

2.4 Discussion

2.4.1 Model simplifications and assumptions

The basic signal obtained for interpretation is the response of the arteriovenous oxygen content difference. The interpretation has been done by a simple oxygen exchange model consisting of two compartments. The parameters estimated are obviously model dependent and relate to the definition of the compartments. The first simplification is the artificial division into compartments with and without oxygen consumption. It has been shown that arterioles and venules exchange oxygen as well. Therefore, most probably there will be a gradual change in capacity of vessels to deliver oxygen to tissue. Hence, in reality a sharp demarcation between exchange vessels and transport vessels is lacking. The second simplification is the assumption that only the oxygen exchange compartment is well mixed, whereas the distal compartment is not. We believe that the conceptual justification for this distinction lies in the contribution of intercapillary shunting of oxygen by diffusion and the many capillary anastomoses. The results of Levitt (1971) and Ziegler and Goresky (1971) support this simplification. Undoubtedly, mixing also occurs more or less continuously in the distal compartment when blood flow merges in the small and larger veins. This mixing effect is neglected by the assumption for the second compartment. The third simplification of the model is the perfusion of the myocardium, being homogeneous in the model, while in reality myocardial
perfusion is quite heterogenous.

We have tried to assess the possible dependency of the estimated volumes on flow heterogeneity and mixing in the distal vessels in the following way. The myocardium was thought to exist of 9 parallel units, each unit consisting of two compartments, the first well-mixed and with oxygen consumption, the second nonmixed and without oxygen consumption. The outlets of the units were connected and hence outflows were mixed. The flow distribution to the units was based on the measurements by Bassingthwaighte et al. (1989) in sheep. This experimental flow distribution, having a relative dispersion (RD=SD/mean) of 29.4 %, was approximated by a histogram defining the amount of tissue having a certain amount of flow (Figure 3.2). Flow was expressed as fraction of the mean, varying between 0.2 and 1.8 with steps of 0.2. Thus each unit was defined by one of the nine flow levels and the fraction of total myocardium having that flow according to the histogram. The response of the AVo₂ on a flow step was then simulated. This response from the distributed model was consequently fitted by our original two compartmental model and the mixed and unmixed volumes and their sum were estimated. This resulted in an overestimation of the total volume by 7 % and mixed volume by 14 %, while unmixed volume was underestimated by 10 %. These deviations between estimated and preset volumes of the units were independent of flow. From these simulations we conclude that the heterogeneity of flow and mixing of blood in the distal compartment are not major sources of error.

Obviously, the volume distribution established by our procedure remains model dependent. However, the assumptions made are not specific for this study. Hence, the parameters found in this study may be very useful for theoretical and experimental studies in which similar assumptions are made (Dankelman et al., 1989a).

The total coronary volume changes caused by flow steps as estimated from the integrated difference of arterial and corrected venous flow were less than 5 % of the sum of mixed and unmixed volumes. Therefore, the assumption that the compartment volumes were constant during the intervention is defensible.

The time course of the O₂ consumption is unknown but was assumed to be exponential. The effects of other possible forms of the time course were not investigated. However, we assumed that effects of variation in the time course deviating from exponential will be within the range of time constants of oxygen consumption changes considered. This systematic effect on the mixed volume was, on average, only about 15 % and on the unmixed volume it was even less.

To obtain the coronary vessel volume of the mixed compartment, a correction for the contribution of tissue to the volume of this compartment was made. The assumptions on tissue volume and linearization of hemoglobin and myoglobin oxygen saturation curves are not crucial for this correction. In the first
place the correction term taking into account these effects is only in the order of 15 %, as explained in the methods section. The uncertainty of the effect of linearization is related to the uncertainty in oxygen pressure. Taking the range of venous oxygen pressures measured we found a maximum error in this correction of 12 %. Hence, the error in estimated volumes is maximally 1.8 %.

2.4.2 Comparison with data on volume from other studies

It has been well established that intramyocardial blood volume is not constant, but depends on arterial pressure, flow and heart contraction (Judd and Levy, 1991, Kajiya et al., 1986, Morgenstern et al., 1973, Salisbury et al., 1961, Spaan, 1985, Wu et al., 1992). Therefore, one has to be careful in comparing estimates of intramyocardial volume from different studies: the circumstances during the measurements may vary. To date, only total vascular volume has been reported for the intact beating heart. These values can be compared with the sum of our mixed and unmixed volume plus an estimate of the volume in the larger arteries. Data on arterial volume were reviewed by Spaan (1985) and a rough estimate of 1.6 ml/100 g was reported for arteries larger than 200 μm. Because oxygen exchange and mixture will probably occur in arteries smaller than 200 μm, we assumed that the volume of these small arteries is included in our estimate of mixed volume. Assuming a value of 1.6 ml/100 g for the arterial volume, total blood volume in the hearts examined by us amounted to 15.3 ± 1.3 ml/100 g with vasomotor tone intact (mean ± SE, n=9) and 19.7 ± 1.5 ml/100 g with vasodilation (n=5). For isolated dog hearts, a value of 12-15 ml/100 g was found from measurements of weight changes in combination with a residual blood volume estimation (Salisbury et al., 1961). In different studies, use of the dye dilution technique resulted in 15.6 ml/100 g (Hirche and Lochner, 1962) and 11.0-17.8 ml/100 g (Morgenstern et al., 1973). The increase in volume by vasodilation as found in this study is consistent with the effect of flow on volumes as found by CT imaging: 8.9 and 11.7 ml/100 g at flows of 1.4 and 2.5 ml/s/100 g, respectively (Wu et al., 1992). In absolute numbers, however, our estimates of total volume are somewhat larger than those estimated by this CT study. In a different CT study, a value of 16.4 ml/100 g was found during vasodilation (Canty et al., 1991). In part, the difference between our data and those of the CT studies can be explained by the volume of the larger vessels which is excluded in the CT studies.

For estimates of volume distribution no data obtained under physiological conditions are available in literature. Reports on small vessel volume, including capillaries and to a variable degree arterioles and venules, vary between 4 ml/100 g (Eliaisen et al., 1982) and 27 ml/100 g (O'Keefe et al., 1978). The volume of the mixed compartment in this study is larger than most previously
reported data. To a large extent, this can be attributed to the perfusion pressure. This pressure was normal in our measurements, but it is often decreased in studies in which volumes were estimated using radioactively labelled red blood cells and/or plasma. Certainly, firmly pressing the heart (Crystal et al., 1981, Weiss and Winbury, 1974) after excision will further reduce vessel volume and may explain the relatively small values found for capillary volume. It may even explain the fact that in these studies no effect of vasodilation on capillary volume was found. The labelling technique combined with freezing of the heart was used in rats to investigate the effect of heart contraction on coronary volume (Judd and Levy, 1991): for small vessel blood volume values of 7.4 and 4.6 ml/100 g were measured during diastolic and systolic arrest, respectively. However, in this study perfusion pressure was also decreased before freezing. In morphological studies on coronary volume, perfusion pressure is often kept within the physiological range during fixation. However, the effects of the method of fixation and the subsequent histological techniques on the absolute volumes are hard to determine. Thus, although these labelling and morphological techniques may be useful in studies of regional volume distributions and the effects of interventions on vessel volumes, the comparison between studies is almost impossible. In any case, our data are well within the range reported but support the high values of microvascular volume.

The unmixed compartment is distal of the $O_2$ exchange vessels, so it must be compared with venous vessel volumes from literature. Judd and Levy (1991) reported the sum of the volumes of arteries and veins larger than 100 µm in rats to be 1.2 ml/100 g during diastolic arrest. This is small compared with our data. However, it is doubtful whether volumes of major vessels between hearts of such different sizes may be compared. Hyde and Buss (1986) and O'Keefe et al. (1978) examined coupes from dog hearts by microscope and reported a venous volume of 2.6 ml/100 g and a large vessel volume, including arteries, of 4.9 ml/100 g respectively at maximal vasodilation. Again, the reason that our estimates of unmixed volume are larger than these values can be attributed to the difference in perfusion pressure.

2.4.3 Implications

Our primary aim was to estimate the distribution of volume between a compartment responsible for oxygen exchange and the compartment distal of it. The method was successful but not sensitive enough to reveal the dependency of the volume distribution on several factors as perfusion pressure or coronary flow over the range in which these were varied. However, the effect of abolishing vasomotor tone was significant. As discussed, this is consistent with the effect on total blood volume as reported by others. Hence, as a first conclusion of our
study, we may state that the increase in total blood volume induced by vasodilation must be looked for in the capillary bed and not in the coronary veins. Obviously, we can not discriminate between all the capillaries increasing their volume by a similar amount or the opening of more capillaries as suggested by theories on capillary recruitment. A second conclusion relates to the time constants involved in reaching equilibrium of tissue oxygen tension at relatively constant metabolism. Such a time constant follows from the ratio between the oxygen stored in blood and tissue and the oxygen supply, which is represented by the ratio of uncorrected mixed volume and coronary arterial flow. In our experiments, the values of 8.3±0.9 and 6.5±0.5 s (mean±SE) for control and vasodilation, respectively, can be calculated for these time constants.
3 Comparison of different oxygen exchange models

Part of this chapter is printed as:

**Classical Krogh model does not apply well to coronary oxygen exchange**
Catharina P.B. Van der Ploeg, Jenny Dankelman, Jos A.E. Spaan

*Oxygen transport to tissue* **XV**, 299-304, 1994

A functional distribution of coronary volume can be estimated from the response of arterio-venous $O_2$ content difference ($\Delta V O_2$) to a flow step. However, the results depend on the assumed oxygen exchange model. The previously used model consisted of a single mixed compartment with oxygen exchange in series with an unmixed compartment without oxygen exchange (reference model, Chapter 2). The purpose of this study is to provide an estimate of the errors made in the volume estimations by not taking into account factors as flow heterogeneity, different mixing sites or Krogh-like oxygen exchange.

The approach is indirect: the response of the $\Delta V O_2$ to a flow step has been calculated with alternative oxygen exchange models in which the mentioned factors were incorporated. These transients have been treated as experimental signals and fitted with the reference model. The resulting estimated volumes are different from the volumes assumed in the alternative models. Large differences are obtained with some of the alternative models, e.g. the model with Krogh characteristics. However, these models seem unrealistic because capillary $P O_2$ is larger than venous $P O_2$. Only small differences in volumes are obtained with the more realistic models. Therefore, these results indicate that the coronary volumes are approximated well by the estimations obtained with the reference model. These volume estimations were 9.9 and 3.8 ml/100 g for the $O_2$ exchange vessels and the distal venous volume, respectively (Chapter 2).

3.1 Introduction

In the previous chapter, the intramyocardial blood volume involved in oxygen exchange and the volume of the coronary vessels distal to these oxygen exchange vessels were estimated from the transient in arteriovenous oxygen saturation difference induced by a sudden flow step. Estimation of these volumes is dependent on the assumed oxygen exchange model. The applied model was based on the assumption of a well-mixed compartment in which average blood $O_2$ pressure ($P O_2$) is essentially equal to venous $P O_2$. This model is only a global approximation of the coronary circulation, which does not take into account the complex capillary anatomy and all convective and diffusive pathways. There are several aspects by which this model may oversimplify reality. The assumption
of mixing is based on rapid diffusion between capillaries and many intercapillary anastomoses. However, blood travels a certain distance through capillaries. This may be better approximated by a number of well-mixed compartments with equal oxygen exchange in series. Furthermore, since there are many bifurcations in the venular system, some mixing of blood may occur there as well. The effects of these possible deviations of the basic assumptions were studied by considering two mixed compartments in series with a distal unmixed compartment. In contrast to the series array model, the two mixed compartments could have different mixing volumes or oxygen exchange. Another aspect to be considered is flow heterogeneity. Especially in the myocardium there may be considerable flow differences between subendocardium and subepicardium. The possible effect of flow heterogeneity on our volume estimates was assessed by analyzing compartments in parallel.

Thus, the purpose of this study is to provide an estimate of the errors made in the volume estimations by not taking into account the factors considered above. Since these factors cannot be altered experimentally, the only way to assess the possible influence is via a model study. Therefore, models in which the different factors are taken into account were compared with the original model, which served as a reference. This original model is the model used for estimating volumes in our experimental study (Chapter 2). With the alternative models, transients in arterio-venous oxygen content difference induced by flow steps were predicted. These transients were treated as experimental signals and fitted by the reference model. In this way, the magnitude of errors to be expected from the assumptions in the reference model could be obtained.

3.2 Methods

3.2.1 Description of the models

All models were based on the mass balance of oxygen. In the simulations flow was changed stepwise and compartment volumes, oxygen consumption and arterial oxygen content were assumed to be constant in time. Since the arterial \( O_2 \) content, \([O_2]_a\), is constant, the responses of the arteriovenous oxygen content difference and the venous \( O_2 \) content, \([O_2]_v\), have the same shape \( (AVo_2(t) = [O_2]_a - [O_2]_v(t)) \), only the direction of the change and offset are different.

Reference model

The reference model consists of a single mixed compartment with oxygen exchange in series with an unmixed compartment without oxygen exchange (Figure 3.1A). For the mixed compartment, the time response of the \( O_2 \) content
COMPARISON OF OXYGEN EXCHANGE MODELS

to a flow step can be calculated on basis of the $O_2$ mass balance:

$$V_m \cdot \frac{d[O_2]_m(t)}{dt} - Q_a \cdot [O_2]_a - Q_a \cdot [O_2]_m(t) - MVO_2(t) \quad \{3.1\}$$

where:
- $V_m$ = volume of the mixed compartment
- $[O_2]_m$ = $O_2$ content in mixed compartment
- $[O_2]_a$ = arterial $O_2$ content
- $Q_a$ = arterial flow
- $MVO_2$ = myocardial $O_2$ consumption
- $t$ = time

With the oxygen consumption being constant, the response of the $O_2$ content from a single mixed compartment after a step in flow is exponential. When the flow after the flow step ($Q_{n,2}$) is constant, the time constant of the exponential response is determined by the volume of the mixed compartment ($\tau = V_m/Q_{a,2}$). The $O_2$ content in the mixed compartment starts to change at the same moment as the change in flow. However, when measured responses of the arteriovenous oxygen content difference ($AVO_2$) are examined, a time delay is apparent (Figure 2.3). This time delay was interpreted as being the result of venous transport vessels, which were included in the model as an unmixed compartment distal of the oxygen exchange part of the model. The volume of this compartment, probably representing the larger veins, was estimated from the time delay ($\tau_d$) and the flow after the flow step: $V_{unm} = \tau_d \cdot Q_{a,2}$. Summarizing, the response of the reference model to a flow step is an exponential curve with a time delay compared to the arterial flow step.

**Series array of mixed compartments with equal volume and oxygen exchange**

A model consisting of $n$ mixed compartments in series has been developed to approximate blood flow through capillaries (Figure 3.1B). As in the reference model, the distal veins are represented by an unmixed compartment without oxygen exchange. The volumes of the $n$ mixed compartments are equal. Oxygen consumption is homogeneously distributed over these compartments. Of each mixed compartment $j$, the $O_2$ content time course can be calculated by:

$$\frac{V}{n} \cdot \frac{d[O_2]_{m,j}(t)}{dt} - Q_a \cdot [O_2]_{m,j-1}(t) - Q_a \cdot [O_2]_{m,j}(t) - \frac{MVO_2(t)}{n} \quad \{3.2\}$$
A.

![Diagram A](image)

B.

![Diagram B](image)

where:

\[ [O_2]_{m,j} = O_2 \text{ content of the } j^{th} \text{ compartment} \]
\[ j = 1 \ldots n \]
\[ [O_2]_{m,j-1}(t) = [O_2]_a \text{ for } j=1 \]

The unmixed compartment causes a time delay. Hence, the output \([O_2]_a(t)\) of the model equals the time-shifted response of the last compartment. Note that the single mixed compartment model is a special case of the series array model: for \(n=1\) both models are equal.

The main distinction between the series array model and reference model is the distribution of oxygen pressure. While in a single mixed compartment \(P_{O_2}\) is distributed homogeneously, in an array of mixed compartments with oxygen consumption the \(P_{O_2}\) declines in the direction of the flow. In this respect the latter model resembles the classical Krogh model (Krogh, 1919).

**Model with two mixed compartments having different oxygen exchange properties**

A model consisting of two mixed compartments in series and an unmixed compartment was developed to account for the possibility of an extra mixing site (Figure 3.1C). Thus, the reference model was extended with one mixed compartment. The ratio of oxygen consumption between the two compartments could be altered. Hence, this model is different from the previous alternative
Figure 3.1 Models. A. Reference model with a single mixed compartment with oxygen exchange and an unmixed compartment without oxygen exchange. B. Series array of mixed compartments and an unmixed compartment. Volumes and oxygen consumption are equal in all mixed compartments. C. Two mixed compartments with oxygen exchange in series with an unmixed compartment without oxygen exchange. D. Model with flow heterogeneity. This model consists of nine parallel paths, each consisting of a mixed and an unmixed compartment. Each path has a different flow and represents a different amount of tissue. The response of the total model to a flow step is calculated from the responses of the individual paths after weighing to flow and amount of tissue with that flow. \( V \): volume, \( V_m \): volume of the mixed compartment, \( V_{unm} \): volume of the unmixed compartment, \( MVO_2 \): myocardial \( O_2 \) consumption, \( Q_A \): arterial flow, \( [O_2]_a \): \( O_2 \) content.
model with \( n = 2 \), where volumes and oxygen exchange from all mixed compartments were equal. The oxygen content time course for the two compartments was calculated, again based on the mass balance for oxygen:

\[
\begin{align*}
V_A \cdot \frac{d[O_2]_A(t)}{dt} & = Q_a \cdot [O_2]_a - Q_a \cdot [O_2]_A(t) - MVO_{2,A}(t) \\
V_B \cdot \frac{d[O_2]_B(t)}{dt} & = Q_a \cdot [O_2]_A(t) - Q_a \cdot [O_2]_B(t) - MVO_{2,B}(t)
\end{align*}
\]  

\{3.3\}

where:

- \( A \) or \( B \) = subscript referring to first or second compartment, respectively
- \( V \) = volume of a mixed compartment
- \([O_2]\) = \( O_2 \) content
- \([O_2]_a\) = arterial \( O_2 \) content
- \( Q_a\) = arterial flow
- \( MVO_2 \) = myocardial \( O_2 \) consumption

The unmixed compartment only causes a time delay. Hence, the output \([O_2]_v(t)\) of the model is the delayed response of the second mixed compartment.

The mixed volumes \( V_A \) and \( V_B \) were varied from 1 to 12 ml/100 g and the responses were calculated and fitted for all 144 combinations. Five different distributions of oxygen consumption between the two mixed compartments were used. Total oxygen consumption from the two compartments had a constant value of 60 \( \mu \)l \( O_2/s/100 \) g. The responses to flow steps from 1.2 to 1.4 ml/100 g were simulated. The effects of flow level and relative flow step were also evaluated.

This alternative model has many parameters that affect the response of venous oxygen content to a flow step. Insight into the effect of these parameters may be provided by the model transfer function. Therefore, the transfer function of this alternative model was calculated after linearization of Equation 3.3. The unmixed compartment does not change the shape of the model response but only causes a time delay: \([O_2]_v(t) = [O_2]_B(t - \tau_d)\). Hence, inclusion of this compartment in the transfer function complicates this function without providing more insight. Therefore, this compartment is not included in the calculation of the transfer function. Applying Laplace transformation yields:

\[
H(s) = \frac{\Delta [O_2]_v(s)}{\Delta Q_a(s)} = \frac{MVO_{2,A} + MVO_{2,B}}{Q_a}, \left( \frac{\frac{V_A \cdot MVO_{2,B}}{Q_a} \cdot \left( \frac{V_A \cdot MVO_{2,B}}{Q_a + 1} \right)}{} \right)
\]  

\{3.4\}
where:
\[ \begin{align*}
Q_{a,0} & = \text{the arterial flow value in the working point} \\
\mathcal{S} & = \text{Laplace operator} \\
\Delta & = \text{the variations of the signal around the working point}
\end{align*} \]

**Model with flow heterogeneity**

Flow heterogeneity was incorporated in a model by assuming that the coronary circulation consists of parallel paths with nine different flows (Figure 3.1D). Each path represents a different amount of tissue and is assumed to consist of a mixed compartment with oxygen consumption in series with an unmixed compartment without oxygen consumption. The inlets and outlets of the paths were connected. The nine flow values were divided evenly around the average flow: the flow of a path, normalized per 100 g tissue, was either 0.2, 0.4, 0.6, 0.8, etc. to 1.8 times the total flow \( Q_a \). For each path the response of the arterio-venous oxygen content difference to a flow step was calculated, assuming a certain mixed and unmixed compartment volume and oxygen consumption. For obtaining the response of the total model, the responses of the different paths were summed after weighing with path flow and amount of tissue represented by the path:

\[
AV_{o_2,\text{tot}}(t) = \sum_{i=1}^{9} w_{t,i} \cdot w_{q,i} \cdot AV_{o_2,i}(t)
\]

\[\text{(3.5)}\]

where:
\[\begin{align*}
i & = 1, 9 \\
w_{t,i} & = \text{weighing factor for the amount of tissue per path} \\
w_{q,i} & = \text{weighing factor for flow, being the ratio of flow in the } i^{th} \text{ path to total flow}
\end{align*}\]

Weighing with path flow was necessary because paths with higher flows contributed relatively more to the \([O_2]_r\), response of the total model than paths with lower flows.

Experimental data on the distribution of flow has been reported by Bassingthwaighte et al. (1989). Regional flow data were obtained from the amount of microspheres trapped in regions of the myocardium. It was found that the relative dispersion (RD, RD=SD/mean) of a regional flow distribution increased when the sample size decreased. From their data, we used the flow distribution obtained with the smallest sample size (0.22 g) from 11 sheep hearts. This flow distribution had a relative dispersion of 29.4% (Figure 3.2). The \(x\)-axis of the histogram was divided in 9 similar parts, and the relative amount of tissue \(w_t\) having a flow of 0.2, 0.4, ..., 1.8 times the average flow was read from the \(y\)-axis.
Figure 3.2 The regional flow distribution (solid curve) as given by Bassingthwaighe et al. (1989) was used to determine the relative amount of tissue having a certain regional flow (dashed lines). The flow distribution is slightly asymmetrical, however, a symmetrical flow distribution was used in the calculations. The curve is the average of 11 sheep hearts. Data are obtained from samples with an average weight of 0.22 g.

Note that in essence, the model represents an infinite amount of parallel paths, each with one of nine discreet flow values. The tissue weighing factor of a path represents the percentage of tissue having a certain flow. In the model, all paths with the same flow per 100 g are assumed to have the same mixed and unmixed volumes and oxygen consumption per 100 g.

The effect of heterogenous flow distribution and distal confluence of blood with different O₂ saturations on the volume estimations was described briefly in Chapter 2. In this chapter we will analyze these effects more extensively.

3.2.2 Fitting procedure

The influence on the volume estimations of the assumed oxygen exchange model was investigated by fitting the reference model response to responses from the alternative models. The response of the reference model is an exponential curve with a delay time. The best fit was obtained by varying the time delay and the time constant of the exponential curve. The moments at which the response of the alternative model had changed relatively 10 and 90 % determined the time period for which the sum of squared differences between alternative model response and exponential curve was minimized. With this method, the estimated mixed volume \( V_m \) could be calculated and compared with the volumes of the mixed compartments of the alternative model. Furthermore, from the time delay \( \tau_d \), the estimated unmixed volume \( V_{unm} \) could be calculated by multiplication of time shift with flow after the flow step: \( V_{unm} = \tau_d \cdot Q_{a,2} \). This volume was compared with the unmixed volume of the alternative model.
The same fitting procedure as described here was also used for the estimation of the coronary volume distribution from the experimentally measured response of the arteriovenous oxygen content difference after a flow step (Chapter 2).

3.3 Results

3.3.1 Series array of mixed compartments with equal volume and oxygen exchange

The response of the series array model depends on the number of compartments n (Figure 3.3). Obviously, when n=1 the series array model is equal to the reference model and the response is exponential with $\tau = \frac{V_m}{Q_{a,2}}$. The addition of compartments results in a deviation of the exponential curve: with many mixed compartments in series the change of venous oxygen content in time becomes almost straight. The slope of this change depends on the volume assumed in the model and the flow.

The response of the distributed model with n=30 and the exponential curve fitted to it are shown in Figure 3.4. Fitting of the single mixed compartment response to the distributed model response results in an underestimation of the oxygen exchange volume. The magnitude of this underestimation depends on the number of mixed compartments in series n (Figure 3.5A). For n=30 the response of the distributed model resembles the response of a continuously distributed model (n=∞). For this model the estimated mixed volume is about 50% of the total volume in the distributed model. The unmixed volume of the series array model was 0 ml/100 g. Therefore, no time delay is present in the series array model response. However, fitting with an exponential curve resulted in an

![Figure 3.3](image.png) Model responses of the venous oxygen content $[O_2]_v$ to a flow step. The response of the single mixed compartment model (n=1) is exponential. Increment of the number of compartments (n) results in a more straight response. $V=10$ ml/100 g, $V_{w,m}=0$ ml/100 g, $MVO_2=60$ μl $O_2$/s/100 g, $Q_a$ from 1.2 to 1.4 ml/s/100 g.
Figure 3.4 The response of a model with a single mixed compartment and an unmixed compartment is fitted to the response of the distributed model (n=30).

Figure 3.5 Volumes found by fitting an exponential curve to the response of a series array of n mixed compartments with V=10 ml/100 g and V_{unm}=0 ml/100 g.
A. Oxygen exchange volume V_n. Oxygen exchange volume appeared to be underestimated. B. Difference in unmixed volume between the two models. Unmixed volume is overestimated.

apparent time delay (Figure 3.4). From this time delay, the difference in unmixed volume between the two models was calculated. Because the flow in both models is equal and homogenous, this unmixed volume difference does not depend on the initial unmixed volume: a larger unmixed volume in the series array model will result in a larger time delay of the fitted response, but a similar difference in time delay between the two responses will be estimated. The unmixed volume was overestimated by 10% of the total mixed volume in the series array model (Figure 3.5B). These error percentages for mixed and unmixed volume were independent of the total volume of the distributed model (varied from 10 to 20 ml/100 g), oxygen consumption (varied from 40 to 120 μl O2/s/100 g) and flow (flow before the flow step was 1.2 ml/s/100 g, flow after the flow step was varied from 1.4 to 3.0 ml/s/100 g and vice versa).
3.3.2 Model with two mixed compartments having different oxygen exchange properties

The model was evaluated with respect to the effect of variation in oxygen consumption distribution and volume distribution between the two mixed compartments. Examples of responses of the venous oxygen content to a step change in flow are depicted in Figure 3.6. Each panel represents a different distribution of oxygen consumption between the two mixed compartments. The four responses within each panel are calculated for combinations of \( V_A \) and \( V_B \) being 1 or 12 ml/100 g. The shape of each response depends on the volumes of the mixed compartments and also on the amount of oxygen consumption from each compartment. Note that, in the first and last panel, different combinations of mixed compartment volumes result in similar responses. The accompanying results of the fitting procedure are shown in Table 3.1. This table shows the estimated \( V_m, \Delta V_{unm} \) and total estimated volume \( V_m + \Delta V_{unm} \). Similar to the series array model, the estimated difference in unmixed volume between the model with two mixed compartments and the original model was independent of the initial unmixed volume (see Section 3.3.1).

![Figure 3.6](image)

**Figure 3.6** Model responses of the venous oxygen content \([O_2]_v\) to a flow step. Responses for four volume distributions have been drawn: solid lines: \( V_A=1, V_B=1 \), dashed lines: \( V_A=1, V_B=12 \), dotted lines: \( V_A=12, V_B=1 \), dash-dotted lines: \( V_A=12, V_B=12 \). Oxygen consumption distribution between the two mixed compartments is different for each plot: from panel A to E \( MV_{O2,A} \) increases and \( MV_{O2,B} \) decreases. The accompanying fitting results are given in Table 3.1.
Table 3.1 Results of fitting the responses from the original two compartment model to the responses from model with three compartments. The results for the four combinations of $V_A$ and $V_B$ being 1 or 12 ml/100 g are presented. Five different oxygen consumption distributions are applied. See Figure 3.6 for the accompanying responses. $V_m$: estimated mixed volume, $\Delta V_{unn}$: estimated difference in unmixed volume between the two models.

<table>
<thead>
<tr>
<th></th>
<th>$V_m$</th>
<th>$\Delta V_{unn}$</th>
<th>$V_m+\Delta V_{unn}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A MVo$<em>{2,A}=0$, MVo$</em>{2,B}=60$</td>
<td>$V_A=1$</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>$V_A=12$</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>B MVo$<em>{2,A}=15$, MVo$</em>{2,B}=45$</td>
<td>$V_A=1$</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>$V_A=12$</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>C MVo$<em>{2,A}=30$, MVo$</em>{2,B}=30$</td>
<td>$V_A=1$</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>$V_A=12$</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>D MVo$<em>{2,A}=45$, MVo$</em>{2,B}=15$</td>
<td>$V_A=1$</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>$V_A=12$</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>E MVo$<em>{2,A}=60$, MVo$</em>{2,B}=0$</td>
<td>$V_A=1$</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>$V_A=12$</td>
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<td>20</td>
</tr>
</tbody>
</table>

Responses of the model with MVo$_{2,A}=0$ are given in Figure 3.6A. It may be obvious, that when no oxygen is consumed from the first mixed compartment, this compartment will not affect the oxygen content response (see transfer function, Equation 3.4). In this case, the model is equivalent to the reference model with one mixed compartment, and the response of the model will be exponential with a time constant corresponding to $V_B/Q_{a,0}$. Thus, the responses with $V_A=1$ and with $V_A=12$ are equal. In Table 3.1 the fitting results with MVo$_{2,A}$ being zero (case A) are shown for the four combinations of $V_A$ being 1 or 12 and $V_B$ being 1 or 12. As expected, the estimated $V_m$ is exactly equal to $V_B$ and $\Delta V_{unn}$ is zero for all volume combinations. The results are independent of $V_A$.

When the oxygen consumption in the second compartment is zero (MVo$_{2,B}=0$, case E), the model response is similar to the response of a series connection of two first order systems, one with a time constant of $V_A/Q_{a,0}$ and one with a time constant of $V_B/Q_{a,0}$ (see transfer function, Equation 3.4). Thus, the precise form of the resulting S-shaped curve depends on the volumes of both
mixed compartments. Exactly similar responses will be obtained when the volumes of the two compartments are reversed. Responses of the model with MVo$_{2,B}$=0 are illustrated in Figure 3.6E, the accompanying fitting results are shown in table 3.1 (case E). With MVo$_{2,B}$ being zero, the sum of estimated V$_m$ and V$_{unm}$ appeared to be almost equal to the sum of V$_A$ and V$_B$ (bottom of Table 3.1): the average overestimation for all 144 combinations of compartmental volume was only 3 ± SD 4 % of the total volume, with a minimal and maximal error of -15 and +10 %, respectively. The volume of the extra mixed compartment B was recovered in both V$_m$, which was larger than V$_A$, and in V$_{unm}$.

When oxygen is consumed from both compartments, the transfer function of the model consists of two integrating terms with time constants V$_A$/Q$_{a,0}$ and V$_B$/Q$_{a,0}$ and a differentiating term with time constant (V$_A$-MVo$_{2,B}$)/(Q$_{a,0}$·(MVo$_{2,A}$+MVo$_{2,B}$)) (Equation 3.4). From this function it is clear that reversal of the volumes of the two compartments will result in a different response (Figure 3.6B,C,D). The fitting results showed that the amount of underestimation of the total volume V$_A$+V$_B$ depends on the relative amount of oxygen consumption from the first compartment (Table 3.1, case B, C and D). With low oxygen consumption from the first compartment, this compartment only moderately modifies the response. Increase of the volume V$_A$ of the first compartment results in only slightly higher estimates of total volume (case B, last column). Hence, the sum of estimated V$_m$ and ΔV$_{unm}$ is smaller than the sum of V$_A$ and V$_B$ (bottom of Table 3.1), because a large fraction of V$_A$ is not incorporated in the estimated volumes. Contrarily, when relative oxygen consumption from the first compartment is high, only a small fraction of the volume of the first compartment V$_A$ is not recovered in the sum of V$_m$ and ΔV$_{unm}$. This reasoning can be extended for other oxygen consumption distributions in the alternative model. In general, the smaller the relative oxygen consumption from the first compartment, the smaller the fraction of the volume V$_A$ of this compartment incorporated in the sum of estimated V$_m$ and ΔV$_{unm}$ and hence the larger the underestimation of total volume V$_A$+V$_B$.

The results were independent of flow level and relative flow step.

3.3.3 Model with flow heterogeneity

Model responses

In Figure 3.7 the responses of the AVo$_2$ to a flow step of 1.2 to 1.4 ml/s/100 g is drawn for the paths with flows normalized per 100 g of 0.4, 0.8, 1.2 and 1.6 times the total flow. For each path i, mixed volume was 10 ml/100 g, unmixed volume was 3.8 ml/100 g and oxygen consumption was 60 μl O$_2$/s/100 g. Each path shows an exponential response to the flow step, with
a time constant $V_{m,i}/Q_i$ and a time delay $V_{ unm,i}/Q_i$. Because of the different delay times of the paths, the response of the total model does not change instantly, but shows a slow initial change. Hence, in contrast to the models in the previous sections, in the heterogenous model the unmixed volume does not simply cause a time delay, but affects the time course of the response. The complete response of the model is illustrated in the last panel of Figure 3.7. This response is obtained by weighing to flow and relative amount of tissue with that flow. Due to the different delay times and time constants, the total response is S-shaped. For
COMPARISON OF OXYGEN EXCHANGE MODELS

comparison, the response of the reference homogenous model to the same flow step is plotted in the same panel.

Results of fitting

Fitting of the responses from the reference homogenous model to the response drawn in Figure 3.7 resulted in an overestimation of the oxygen exchange volume \( V_m \) by 1.5 ml/100 g (15 %) and an underestimation of the unmixed volume by 0.4 ml/100 g (11 %). Total volume was overestimated by 1.1 ml/100 g (8 %). Variations in \( V_m \) (4-15 ml/100 g), \( V_{unn} \) (1.8-6.2 ml/100 g) and flow (\( Q_{a,before \ step} \) 0.8-3.0 ml/s/100 g, flow change 15 %) resulted in only very small deviations from these values. Variations in the values for oxygen consumption and % flow change essentially did not change the results.

The results described above were obtained with \( V_m \), \( V_{unn} \) and oxygen consumption equal for each path. The effect of different values of these variables for the different paths was also investigated. When oxygen consumption heterogeneity is equal to the flow heterogeneity (0.2, 0.4, etc. to 1.8 times the average value) and the paths with the largest flows were assumed to have the largest oxygen consumption, unmixed volume is underestimated as above (11 %), but the error in mixed volume estimation is zero. A less pronounced variation in oxygen consumption (0.6, 0.7, etc. to 1.4 times the average oxygen consumption) results in a small error in mixed volume (8 %). In a similar way, variations of \( V_m \), \( V_{unn} \) or both results in errors of the volume estimations that are smaller than when the volumes are equal for all paths. Contrarily, when paths with large flows are assumed to have small volumes or oxygen consumption, the errors in estimation of \( V_m \) and \( V_{unn} \) are larger than when these variables are equal for all paths.

3.4 Discussion

Estimations of the coronary vascular volume could be obtained from transients of arterio-venous oxygen exchange after a flow step (Chapter 2). The oxygen exchange model used to analyze these transients was only a global approximation of the coronary circulation and did not take into account several aspects as e.g. distributed oxygen exchange and flow heterogeneity. The purpose of this study was to investigate the effect on the volume estimations of neglecting these aspects. Therefore, models in which these aspects were incorporated were compared to the original oxygen exchange model.
3.4.1 Series array of mixed compartments with equal volume and oxygen exchange

Comparison of the series array of mixed compartments with the reference model revealed that coronary volume estimated from experimental AVo₂ responses with the series array model would have been about a factor two larger than the values estimated by using the reference model with a single mixed compartment. Hence, the results depend to a large extent on the assumed oxygen exchange model. The volume estimated in previous examinations by using the reference model were within the range of coronary volumes which have been previously published, although they were rather high (mixed volume of 9.9 ml/100 g, unmixed volume of 3.8 ml/100 g, see Chapter 2). Coronary volumes that are twice as large seem unrealistic.

Apart from the coronary volume distribution, the models also predict an oxygen pressure distribution. The O₂ pressure distribution of the series array model is considerably different from the Po₂ distribution in the reference model. In the reference model, partial oxygen pressure is distributed homogeneously. Therefore, the Po₂ of blood leaving the compartment (venous Po₂) is equal to the Po₂ in the compartment (average capillary Po₂). However, in the series array model, Po₂ declines gradually in the direction of the flow while blood flows through a capillary. In this respect, the series array model shows the characteristics of a Krogh model (Krogh, 1919). This results in a venous Po₂ being less than the average capillary Po₂. Measurements and model studies revealed that capillary and tissue Po₂ might be less than venous Po₂ (Whalen, 1971; Wieringa, 1985; Honig and Gayeski, 1989; Gayeski and Honig, 1991). These findings indicate that a single Krogh-like cylinder is a less realistic model for coronary O₂ exchange than a single mixed compartment.

Both the results on the coronary volume distribution estimation and the Po₂ distribution indicate that the O₂ exchange is better modelled by a single mixed compartment than by a series array of mixed compartments.

3.4.2 Model with two mixed compartments having different oxygen exchange properties

An extra mixed compartment besides the oxygen exchange vessels may be formed by the venules. Addition of such an extra compartment to the model used for the volume estimations requires the introduction of two extra parameters: the compartment volume and the oxygen consumption distribution between the two mixed compartments. Analysis of measured AVo₂ signals with this model showed that several combinations of parameters resulted in model responses that fitted the measured response about equally well. Hence, a conclusive estimate of the
coronary volume distribution could not be made when an extra mixed compartment was introduced in the model. The effect on the volume estimations of neglecting a possible extra compartment was investigated.

When the venules are represented by the extra mixed compartment, this extra compartment should be modelled between the original mixed and unmixed compartments and only a small amount of oxygen will probably be consumed from it. The simulations showed that in this case the volume of the extra compartment is recovered in the volume estimations. Both the volume of the mixed compartment with oxygen exchange and the volume of the unmixed compartment without oxygen exchange include a part of the volume of the extra compartment. Since the venules most probably behave partially as transport vessels and partially as a mixing site, it may not be erroneous to distribute the venular volume to the two compartment volumes.

Low oxygen consumption from the first of two mixed compartments may occur when the smaller arteries are modelled as an extra mixed compartment. The volume of this extra compartment will probably be small, since arterial volume is much smaller than capillary volume (e.g. Spaan, 1985). The simulations showed that when oxygen consumption from the first compartment is relatively small, the volume of this compartment will be recovered in the estimations of $V_m$ and $V_{unm}$ for only a small part. Hence, the volume of this compartment almost does not contribute to the volume estimations: the arterial volume is not included in the model estimations of the volume of the mixed compartment with oxygen exchange and the volume of the unmixed compartment without oxygen exchange.

A serious error in total volume estimation will occur if the coronary circulation should be represented by two mixed compartments with about similar oxygen consumption in series with an unmixed compartment. However, as with series array model (Section 3.4.1), application of this model would also result in larger volume estimations than those obtained with the reference model. Furthermore, in this alternative model capillary $Po_2$ is again larger than venous $Po_2$. Therefore, the conclusion from the analysis of the model with two mixing sites is that the extra mixing site either causes no substantial error in the volume estimations or that the model is less realistic than the reference oxygen exchange model with only one mixing site.

3.4.3 Model with flow heterogeneity

A measured regional flow heterogeneity was incorporated in a model with parallel paths to evaluate the effect of flow heterogeneity on the volume estimations. Comparison of this model with the reference model resulted in errors in mixed, unmixed and total volume estimations that were on average 14 %,
-11% and 7%, respectively. Oxygen consumption and volumes were equal in all paths to obtain these results. However, flow is often adapted to the metabolic needs of the tissue. Therefore, it may be likely that the paths with relatively high flow also have relatively high oxygen consumption. The unrealistically high arterio-venous oxygen content differences in the paths with low flow also support this. When this aspect is incorporated in the model, the errors in the estimation of mixed and unmixed volume are even smaller. Therefore, we conclude that flow heterogeneity and the confluence of blood with different O₂ saturations in the distal compartment have a minor influence on the volume estimates.

3.5 Conclusions

The reference model used for the estimation of the coronary volume distribution consisted of a single mixed compartment with oxygen exchange in series with an unmixed compartment without oxygen exchange (Chapter 2). Hence, this model is only a very global approximation of the coronary circulation. Comparison with other oxygen exchange models, also being global approximations of the coronary circulation but allowing phenomena as flow heterogeneity, different mixing sites or Krogh-like oxygen exchange, resulted in different volume estimates. It was indicated that some of these models, e.g. the model with Krogh characteristics and the model with two mixing sites from which a significant amount of oxygen was consumed, seemed unrealistic. However, the volume estimations obtained with the more realistic models did not deviate much from the volume estimates obtained with the reference model. Therefore, these results support the idea that the volume estimations obtained with the reference model are realistic. At a heart rate of 90 beats/min these volume estimations were 9.9 and 3.8 ml/100 g for the O₂ exchange vessels and the distal venous volume during control, and 13.1 and 5.0 ml/100 g during maximal vasodilation, respectively (Chapter 2).
Heart rate influences Gregg's phenomenon during flow controlled perfusion in goats

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The effect of a step in coronary arterial flow (Qa) to the myocardial oxygen consumption (MVO₂) was investigated at different heart rates (HR) to further elucidate Gregg's phenomenon.

In six anesthetized goats the left main coronary artery and the great cardiac vein were cannulated. The hearts were paced alternately at 60 and 130 beats per minute. Flow steps were applied at both heart rates during control and maximal vasodilation by adenosine. MVO₂ in steady state before and after the flow step was calculated by multiplication of Qa and arterial-venous oxygen content difference (Fick's law).

HR affected the MVO₂ dependency on flow (ΔMVO₂/ΔQa) during control as well as during maximal vasodilation. With tone present ΔMVO₂/ΔQa in μl O₂/ml was 16.0±3.6 at HR 60 and 21.7±3.9 at HR 130. During maximal vasodilation these values were 9.5±2.9 and 17.0±5.3 at HR 60 and 130, respectively.

The higher MVO₂ dependency on flow at high HR may be explained via a capillary pressure dependency of Gregg's phenomenon. The capillary pressure change induced by a flow step is expected to be larger at high HR than at low HR because of increased venous resistance at high HR due to increased compression by the heart contraction.

4.1 Introduction

In 1963 Gregg reported that an increase in perfusion pressure of the coronary circulation resulted in an increase in myocardial oxygen consumption in dogs. Since then 'Gregg's phenomenon' has been subject of many studies (Feigl, 1983 (review); Miller et al., 1987; Kitakaze and Marban, 1989; Goto et al., 1991; Schouten et al., 1992). Changes of perfusion pressure as well as coronary flow were reported to induce myocardial O₂ consumption changes and changes in contractility. Some recent studies, however, report absence of a Gregg effect in pigs (Miller et al., 1990; Schulz et al., 1991; Schwartz et al., 1992).

Different hypotheses have been presented to explain Gregg's phenomenon. Gregg (1963) already suggested that coronary distension by increased transmural pressure in the capillaries may stretch the myocardial fibers. Via the Frank-Starling mechanism this would result in increased cardiac work and oxygen consumption. This mechanism has later been named the gardenhose effect.
(Arnold et al., 1968).

The role of contractility was focussed on by Kitakaze and Marban (1989). Ferret hearts showed an increase in force of contraction and intracellular Ca\(^{2+}\) transients after an increase in perfusion pressure, whereas the left ventricular segment length was not affected. With a study on the Tyrode perfused papillary muscle of the rat, Schouten et al. (1992) presented additional evidence for this hypothesis. These authors suggested that the effect of perfusion on contractility may be mediated via capillary pressure, since this pressure affects the volume of the interstitium and capillaries. When this extracellular space is small, it may be depleted from calcium-ions during contraction. These Ca\(^{2+}\)-ions are necessary for force development in the myocardial cells. Hence, with decreased capillary pressure, contractility may be decreased due to diminished Ca\(^{2+}\)-transport into the myocytes.

It is difficult to rule out the possibility of improvement of local perfusion after an increase in flow (Feigl, 1983). However, Gregg’s phenomenon has been observed at high flow rates (Abel and Reis, 1970), making this unlikely.

In this study we tried to further elucidate Gregg’s phenomenon by investigating the effect of a step in coronary arterial flow (Q\(_a\)) to the myocardial oxygen consumption (M\(V_o_2\)) at different heart rates (HR) in blood-perfused in vivo goat hearts. Heart contraction impedes flow by increasing microvascular resistance (see Hoffman and Spaan, 1990, for review). With an increased postcapillary resistance due to a higher heart rate, a certain flow increase will result in a larger increase in capillary pressure. Consequently, on the basis of the hypothesis that an increased capillary pressure is the underlying mechanism of Gregg’s phenomenon, one would expect a larger increase in oxygen consumption with a flow increase at higher heart rate. The hypothesis that heart rate affects Gregg’s phenomenon was tested. Furthermore, the coronary distension hypothesis as the underlying mechanism of Gregg’s phenomenon in the whole heart preparation was evaluated by investigating the relation between the amount of oxygen consumption change and coronary volume change due to the flow step.

### 4.2 Methods

#### 4.2.1 Preparation

The preparation and experimental setup are similar to those described in Chapter 2. Six goats weighing 16 to 27 kg were used in the present study. The goats were treated for worms using ivermectine (MSD) two weeks prior to the experiment. The goats were anesthetized by intramuscular injection of a mixture of 3 ml ketamine-hydrochloride (Aescoket, 100 mg/ml), 3 ml Rompun (20
mg/ml) and 4 ml atropine-sulphate (0.5 mg/ml). Anesthesia was maintained by intravenous injection of 30 ml fentanyl (0.05 mg/ml). 2 ml pancuroniumbromide (Pavulon, Organon, 2 mg/ml) was given intravenously for muscle relaxation. The goats were ventilated with a Harvard respirator using a 2:1 nitrous oxide-oxygen mixture.

A left thoracotomy was performed, and the 3rd and 4th ribs were removed. The pericardium was opened and a cradle was formed. The left main coronary artery was dissected and a ligature was placed around it. Another ligature was placed around the great cardiac vein, close to the junction where the left hemiazygos vein drains into the coronary sinus. The His bundle was destroyed by local injection of formaldehyde (37 %) and the right ventricle was paced. A thin catheter was inserted into the left anterior descending vein according to the Herd-Barger technique (1964). A catheter tip manometer (PPG Hellige, model HD 36 010 80001, 6F) was inserted through a purse string in the left atrial appendage into the left ventricle. A stainless-steel Gregg cannula was inserted into the aorta via a purse string after administration of 3 ml heparin (5000 IU/ml iv). Without disruption of coronary flow, the cannula was ligated into the left main coronary artery. The great cardiac vein was cannulated via the left hemiazygos vein. Anticoagulation was maintained by continuous infusion of heparin (5000 IU/hour).

4.2.2 Arterial perfusion/venous drainage system

The arterial perfusion system was essentially similar to the one previously described by Spaan et al. (1981). Blood from the left carotid artery was pumped into a 70 ml reservoir, approximately half filled with blood, via a heat exchanger and filter (Mirospore, 40 μm). A precision pressure regulator (Fairchild, model 10R) connected to a laboratory compressed air system held reservoir pressure at a preset value. Perfusion pressure was measured at the cannula tip with a catheter tip manometer (Millar SPC-350, 5F). An electromagnetic cannulating flow probe, interposed in the perfusion line, monitored coronary arterial flow (Statham, inner diameter 5 mm, model Sp2202). The blood reservoir was equipped with a level controller (M.S.A. automatic suction control) with feedback to the roller pump, in order to keep the blood level in the reservoir constant.

Blood from the cannulated great cardiac vein drained into a similar pressure controlled reservoir via a 2.5 mm flow probe (Statham, model Sp2202). The pressure in the reservoir was regulated at a preset value. Venous blood was pumped back into the left jugular vein via a heat exchanger and filter (40 μm).

The arterio-venous O₂ content difference was measured dynamically with a device similar to the one first described by Shepherd et al. (1975). The original design was modified in order to obtain faster dynamic responses (Vergroesen and
Spaan, 1988). The technique is based on the measurement of the color difference at 660 nm between arterial and venous blood caused by the difference in hemoglobin O₂ saturation. The arterial cuvette was placed in the arterial perfusion system before the pressure controller. The venous cuvette was placed directly after the venous cannula.

4.2.3 Protocol and measurements

Left ventricular, coronary arterial and venous pressure as well as coronary arterial and venous flow and arterio-venous O₂ content difference were continuously recorded on a Hewlett-Packard F.M. instrumentation recorder (HP 3968A) for analog backup. A/D conversion was done on-line at a sample rate of 80 Hz for at least 100 s, starting 15 s before the flow step. Digitized data were stored on hard disk.

The protocol started with a 15 s occlusion of the arterial perfusion line and a 2 s occlusion of the venous drainage line to check for the zeros of the flow probes. This was repeated several times during the protocol. Between the occlusions, the perfusion condition was changed from constant pressure perfusion to constant flow perfusion by placing a resistance on the perfusion line distal to the pressure reservoir and raising the pressure in the reservoir to compensate for the increased resistance. Flow steps were induced with the heart rate alternating between 60 and 130 beats/min by stepwise changing the reservoir pressure between two levels. By adjustment of the reservoir pressures the average flow was changed, and flow steps at the two heart rates were repeated at this different flow level. After these measurements with tone present, which are referred to as "control" throughout this chapter, adenosine was administered to obtain maximal vasodilation. To test for maximal vasodilation, the arterial perfusion line was clamped for 15 s during constant pressure perfusion. If reactive hyperemia had not yet disappeared completely, the intracoronary infusion rate of adenosine was increased. The flow steps at the two average flow levels and the different heart rates were repeated.

pH, arterial Po₂ and Pco₂ were measured every 30 minutes with an automated blood gas analyzer (model ABL330, Radiometer). Hemoglobin content, arterial and coronary venous oxygen saturations were measured with a hemoximeter (model OSM 2, Radiometer) before each intervention. These data were used for calibration of the dynamic arterio-venous O₂ content difference measurements.

The heart was excised at the end of the experiment. A mixture of gelatine and white paint (Latex) at 37°C was injected into the left main coronary artery. After cooling in a freezer for approximately half an hour, the colored tissue could be dissected and weighed.
4.2.4 Quantification of Gregg’s phenomenon

Gregg’s phenomenon was quantified as the change in O₂ consumption due to a change of arterial flow. O₂ consumption was determined in steady state before and after the flow step from the measured flow and arterio-venous O₂ content difference by application of Fick’s law:

\[ \text{MVO}_2 = Q_a \cdot A\text{VO}_2 \]  \hspace{1cm} (4.1)

The effect of flow steps on MVO₂ was quantified by calculation of the O₂ consumption change normalized to the magnitude of the flow change (ΔMVO₂/ΔQ₀). Calculations were done with signals averaged per heart beat. A heart beat was defined as the period between the onset of two subsequent systoles.

The change in contractility due to a flow step was investigated indirectly by examination of the maximum of the derivative of the left ventricular pressure (dPₙ/dt) per heart beat. However, the changes in dPₙ/dt induced by the flow step were small compared to the variations under control conditions. Therefore, we considered these measurements too insensitive to draw any conclusions concerning contractility.

4.2.5 Estimation of change of coronary volume induced by a flow or heart rate change

The change in coronary volume induced by a flow or heart rate change was calculated from the integrated difference between arterial inflow and venous outflow: \[ \Delta V(t) - \int_0^t (Q_a(\tau) - \alpha \cdot Q_v(\tau)) d\tau \] as described by Vergroesen et al. (1987a). The flow from the great cardiac vein Qᵥ is only about 50 % of the flow into the left main coronary artery Qₐ. Therefore Qᵥ had to be corrected by a factor. This factor α, being the ratio of Qₐ and Qᵥ in steady state, causes ΔV(t) to be zero in a period of steady state.

The volume change calculation is disturbed by two phenomena: ventilation and change in outflow distribution (α) due to the flow step. Ventilation caused ΔV(t) to vary periodically. To minimize the effect of ventilation on the determination of volume change, ΔV(t) was calculated over a full number of respiration periods. Outflow distribution often changed slightly due to the flow step, resulting in different steady state values of α before and after the flow step. In order to assess the influence of the change of α, the volume change of the coronary bed was calculated in two ways: 1. with a constant factor α, equal to the Qₐ/Qᵥ ratio before the flow step, and 2. with α changing stepwise from the value in steady state prior to the steady state value after the flow step. The actual volume change is probably an intermediate value.
4.2.5 Statistics

Data were grouped and averaged per animal. Reported data are means ± SE of these averages. Comparisons of paired variables at the two heart rates were performed with a paired two-tailed t-test. A value of P<0.05 was considered statistically significant.

4.3 Results

In Figure 4.1 a typical recording of a flow step is shown. The first 3 s are plotted on a different time scale to show the variation within a heart beat. The flow decrease resulted in a gradual increase in AVO₂ to a different steady level.

Average pressures, flows, heart rate and oxygen consumption were determined before and after each flow step. These values were averaged to obtain one value per flow step irrespective of the direction of the flow step. These data were then grouped according to heart rate and vasodilatory state and averaged per animal. In Table 4.1 the means ± SE of these averages are provided to give an indication of the values as occurred in the study.

Figure 4.2 shows the data of a typical experiment at control (left panel) and maximal vasodilation (right panel). The change in O₂ consumption due to the

<table>
<thead>
<tr>
<th>Table 4.1 Hemodynamic data for control and maximal vasodilation grouped according to heart rate (HR), and heart weight.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td><strong>HR 60</strong></td>
</tr>
<tr>
<td>P_{lv syst} [mmHg]</td>
</tr>
<tr>
<td>P_{p} [mmHg]</td>
</tr>
<tr>
<td>Q₉ [ml/s/100 g]</td>
</tr>
<tr>
<td>Q₉ change [%]</td>
</tr>
<tr>
<td>Q₉ [ml/s/100 g]</td>
</tr>
<tr>
<td>P_{epi ven} [mmHg]</td>
</tr>
<tr>
<td>HR [beats/min]</td>
</tr>
<tr>
<td>MVO₂ [μl O₂/s/100 g]</td>
</tr>
</tbody>
</table>

Heart weight [g]:
- total | 133 ± 11 |
- left ventricle | 80 ± 7 |
- perfused area | 78 ± 7 |

Means ± SE, n = 6 goats. P_{lv syst}: systolic left ventricular pressure, P_{p}: perfusion pressure, P_{epi ven}: epicardial venous pressure, Q₉: coronary arterial flow, Q₉: coronary venous flow, MVO₂: myocardial oxygen consumption.
Figure 4.1 Left ventricular pressure ($P_L$), perfusion pressure ($P_p$), coronary arterial flow ($Q_a$), coronary venous flow ($Q_v$) and arterio-venous oxygen content difference ($A\textVO_2$) recorded during maximal vasodilation at HR 130. At $t=15$ s flow was changed. The dashed lines indicate the moment of the flow step. The first 3 s are plotted on a different time scale to show the variations per heart beat.

flow step is illustrated by connection of the data points measured before and after a flow step. The effect of heart rate on the sensitivity of $O_2$ consumption for flow was assessed by comparison of the slopes of these connecting lines. These slopes are referred to as $O_2$ consumption sensitivity for flow or $\Delta M\textVO_2/\Delta Q_a$. In this goat, $O_2$ consumption dependency on flow was only slightly larger at HR 130 compared to HR 60 when tone was present, whereas it was significantly increased at the higher heart rate during maximal vasodilation.

The $O_2$ consumption sensitivities for flow were grouped according to heart rate and vasodilatory state (4 groups). These sensitivities were averaged per
animal per group. The means over the animals are presented in Figure 4.3. The effect of heart rate on flow sensitivity of O₂ consumption ($\Delta$MV̄O₂/$\Delta$Qₐ) is significant due to the consistency of the direction of the change both during control and maximal vasodilation (P<0.05). Furthermore, during maximal vasodilation also the relative change in oxygen consumption normalized to the flow step was significantly larger at HR 130 than at HR 60: values for ($\Delta$MV̄O₂/MV̄O₂)/$\Delta$Qₐ in 1/(ml/s/100 g) are 0.21±0.03 and 0.25±0.04 for HR 60 and 130 during control and 0.13±0.04 and 0.19±0.05 for HR 60 and 130 during maximal vasodilation, respectively. Comparison of data between control and maximal vasodilation revealed no significant effect of vasodilation on the flow sensitivity of oxygen consumption.

To investigate whether heart rate affects the absolute coronary volume, the coronary volume change induced by a heart rate change was determined. When heart rate was alternated between 60 and 130 beats/min, coronary volume changed 0.38±0.06 ml/100 g during control, whereas during maximal vasodilation coronary volume was 0.79±0.11 ml/100 g larger at HR 60 than at HR 130 (α changed stepwise). The method of volume change determination had almost no effect: with constant α these values were 0.41±0.13 and 0.75±0.12 ml/100 g, respectively. Hence, coronary blood volume is markedly decreased at a higher heart rate.

Coronary blood volume was also affected by flow: an increase in flow resulted in an increase in blood volume (Table 4.2). However, the sensitivity of this volume change to the flow variation, $\Delta$V/$\Delta$Qₐ, was hardly dependent on heart rate and only reached statistical significance when α was kept constant.
Figure 4.3 O₂ consumption (MVo₂) sensitivity for flow (Qₐ). Data are means ± SE of 6 goats. O₂ consumption changes normalized to the flow step are larger at HR 130 than at HR 60 during control and maximal vasodilation. No significant effect of vasodilation on the flow sensitivity of MVo₂ was found. *: P<0.05.

Table 4.2 Coronary volume changes induced by a flow step for control and maximal vasodilation grouped according to heart rate (HR).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maximal vasodilation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 60</td>
<td>HR 130</td>
</tr>
<tr>
<td>Qₐ [ml/s/100 g]</td>
<td>1.6 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Qₐ change [%]</td>
<td>24 ± 3*</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>α [-]</td>
<td>2.18 ± 0.04</td>
<td>2.16 ± 0.07</td>
</tr>
<tr>
<td>α change [%]</td>
<td>-1.9 ± 1.5</td>
<td>-2.2 ± 1.6</td>
</tr>
<tr>
<td>Coronary volume change [ml/100 g]:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- α varied</td>
<td>0.62 ± 0.08*</td>
<td>0.55 ± 0.09</td>
</tr>
<tr>
<td>- α constant</td>
<td>0.47 ± 0.09*</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td>Coronary volume change normalized to flow change (ΔV/ΔQₐ):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- α varied</td>
<td>1.83 ± 0.25</td>
<td>1.79 ± 0.32</td>
</tr>
<tr>
<td>- α constant</td>
<td>1.39 ± 0.24</td>
<td>1.32 ± 0.27</td>
</tr>
</tbody>
</table>

Means ± SE, n=6 goats. α is the ratio of coronary arterial flow (Qₐ) and coronary venous flow (Qᵥ) during steady state. Differences between HR 60 and HR 130 were tested using paired t-test, *: P<0.05.
4.4 Discussion

The effect of perfusion on myocardial oxygen consumption is known since 1963 (Gregg). However, there is no reason to believe that this effect would depend on the oxygen consumption level. Hence, our result that Gregg’s phenomenon is more pronounced at higher heart rates is not immediately explained. At this moment there is no unique explanation for the cause of Gregg’s phenomenon. It has been suggested that capillary pressure may be involved (Section 4.4.3). In that case, a larger \( O_2 \) consumption change due to a certain flow step would be expected at a higher heart rate, because venous resistance is expected to be larger at higher heart rates due to increased venous compression by the heart contraction. Hence, the suggestion that capillary pressure is involved in the mechanism causing Gregg’s phenomenon explains our results.

4.4.1 Limitation of the AVo\(_2\) measurement

The AVo\(_2\) signal was not steady but pulsatile (Figure 4.1). This pulsatility may be due to the effect of flow by either cell sedimentation or cell orientation changes. However, the effect of flow on the time averaged AVo\(_2\) signal appeared to be minimal. There is a short period after the flow step in which the arterial and venous flows reach their new equilibrium before AVo\(_2\) is affected by the altered extraction. In only some cases, as in Figure 4.1, there was an effect of mean flow on AVo\(_2\) in this period. On average, this flow effect was small compared to the total AVo\(_2\) change due to the change in extraction (-1.0 ± 0.8 %, n=6 goats). Therefore, flow sensitivity of the AVo\(_2\) measuring device was considered negligible.

4.4.2 Exclusion of hypoxia as a cause of Gregg’s phenomenon

Gregg’s phenomenon is sometimes thought to be occurring in ischemic hearts only (Feigl, 1983, Schulz et al., 1991). In our experiments coronary blood flow was kept within or above the normal range and venous O\(_2\) saturation was more than 45 % due to the levels of flow imposed. This high value of venous O\(_2\) saturation excludes the possibility of global hypoxia, but it does not rule out the possibility of local myocardial hypoxia. However, Von Restorff et al. (1977) measured a reduction of coronary venous O\(_2\) saturation down to 9 % during heavy exercise in dogs. Vasodilatory capacity was not exhausted under these circumstances, since an additional hypoxic stimulus further increased coronary flow. Therefore, local hypoxia probably did not impede cardiac work and myocardial O\(_2\) consumption in their experiments. It is therefore unlikely that local
hypoxia occurred in our experiments, where venous saturations were much higher.

4.4.3 Capillary pressure as a cause of Gregg’s phenomenon

Decades ago it was already suggested that capillary pressure might be involved in the cascade of events causing Gregg’s phenomenon (Gregg, 1963; Scharf and Bromberger-Barnea, 1973). Proposed mechanisms act via intravascular or extracellular volume. E.g. a change in capillary pressure might cause a change in transmural pressure, which will result in a coronary blood volume change. Therefore, capillary pressure might be responsible for Gregg’s phenomenon via the coronary distension hypothesis (garden hose effect). Furthermore, capillary pressure might also be involved if Gregg’s phenomenon is caused by a change of intracellular Ca$^{2+}$ transients (Kitakaze and Marban, 1989; Schouten et al., 1992). Schouten and colleagues hypothesized that the extracellular space (capillaries and interstitium) may be transiently depleted of its small amount of Ca$^{2+}$-ions during the action potential, when Ca$^{2+}$ flows from the extracellular space into the myocytes. Since the volume of the extracellular space increases with increased capillary pressure, more Ca$^{2+}$ will be available for the Ca$^{2+}$-influx, and thus contraction will be enhanced at higher pressures.

If capillary pressure is involved in the cascade causing Gregg’s phenomenon, the increased O$_2$ consumption sensitivity to flow at HR 130 compared to HR 60 can be explained on basis of the assumption that heart contraction increases venous resistance. Hence, at a higher heart rate the venous resistance is higher. Capillary pressure is determined by venous pressure, venous resistance and coronary flow: $P_{cap} = P_v + Q_a \cdot R_v$. Consequently, the increase in capillary pressure due to an increase in flow will be larger at a higher heart rate.

Some studies have attempted to distinguish between a coronary flow and a coronary pressure effect of Gregg’s phenomenon (Abel and Reis, 1970; Zborowska-Sluis et al., 1977; Scharf and Bromberger-Barnea, 1973). The results are consistent with the role of capillary pressure. One should keep in mind that capillary pressure is more likely related to flow than to perfusion pressure, since arteriolar smooth muscle tone uncouples coronary arterial and capillary pressures. Abel and Reis (1970) measured increased contractility when flow was increased by vasodilation without an increase in perfusion pressure, whereas a perfusion pressure decrease at constant flow had a much smaller effect on contractility. A similar study of Zborowska-Sluis et al. (1977) using myocardial O$_2$ consumption as an indicator of Gregg’s phenomenon demonstrated the same result. It is impossible to change the coronary flow without changing the pressure distribution of the coronary circulation. Therefore it is not possible to separate the effects of coronary flow and perfusion pressure as determinants of Gregg’s phenomenon.
4.4.4 The coronary distension or gardenhose hypothesis

The gardenhose hypothesis suggests that the increased oxygen consumption due to increased perfusion is caused by a change of myocardial fiber stretch. The augmented stretch is assumed to be induced by an enlargement of the blood volume of the coronary vessels. Thus, the change in coronary volume induced by a flow step should correlate to the change in O₂ consumption according to this hypothesis.

A flow step changed O₂ consumption and coronary volume in equal directions. However, O₂ consumption sensitivity to flow was larger at HR 130 than at HR 60, but the volume changes due to the flow step are about equal at HR 60 and HR 130 (Table 4.2). Hence, one might conclude that Gregg’s phenomenon is not caused by coronary distension. However, the relation between coronary volume change and cardiac fiber stretch is far from obvious because the levels of volume are different at different heart rates. Furthermore, the distribution of the coronary volume change over the different vessel types is unknown, but may depend on heart rate. These reasons might cause a difference in magnitude of Gregg’s phenomenon whereas coronary volume changes are equal. Thus, by measuring coronary volume changes a conclusion concerning the coronary distension hypothesis cannot be given.

4.5 Conclusions

Our results show that during constant flow perfusion of in situ working goat hearts, O₂ consumption changes due to flow steps are larger at a heart rate of 130 beats/min than at a heart rate of 60 beats/min. Thus, heart contraction influences the interactions between coronary perfusion and myocardial O₂ consumption. Hence, quantification of Gregg’s phenomenon requires a definition of the mechanical performance of the heart.

Under the assumption that Gregg’s phenomenon is dependent of capillary pressure, an increased venous resistance induced by the heart contraction could explain our results. However, the complex interaction between heart contraction, coronary perfusion, intramyocardial pressure and volume and O₂ consumption and contractility should be investigated further to unravel the precise mechanism causing Gregg’s phenomenon.
5 Transients in myocardial oxygen consumption following abrupt changes in perfusion pressure in the goat

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Submitted for publication

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Transients in oxygen consumption following abrupt changes in perfusion pressure before and after decreasing the rate of regulation by administration of glibenclamide were analyzed. The left main coronary artery of the open chest anesthetized goat was perfused at constant pressure ($P_p$). $P_p$, arterial flow ($Q_a$) and the arterio-venous oxygen content difference ($\Delta V_{O_2}^{-}$) were averaged per beat. The $\Delta V_{O_2}^{-}$ signal was corrected for mixing processes in the capillaries and transport of blood from the capillaries to the venous measuring site ($\Delta V_{O_2}^{*}$). With an increase in $P_p$, the maximal change of myocardial $O_2$ consumption ($M\Delta V_{O_2}=Q_a*\Delta V_{O_2}^{*}$) was 15.0±3.6% (mean±SE) during control and 31.3±2.3% with glibenclamide. With a decrease in $P_p$ these changes were 16.5±2.9% and 21.0±1.1%. During control, $t_{50}$ for the $M\Delta V_{O_2}$ response was 4.2±0.8 s for an increase and 4.0±1.0 s for a decrease in $P_p$. With glibenclamide these values were 16.9±2.2 s and 22.6±2.0 s. Also $t_{50}$ values for resistance changes were increased with glibenclamide. It is concluded that vasomotor tone diminishes the pressure induced oxygen consumption (Gregg's phenomenon).

5.1 Introduction

Myocardial oxygen consumption is affected by alterations in perfusion pressure (Gregg, 1963). This so-called 'Gregg's phenomenon' has been found in many studies (e.g. Arnold et al., 1968; Gregg, 1963; Kitakaze and Marban, 1989; Schouten et al., 1992). The mechanism by which perfusion alters oxygen consumption is unknown (Feigl, 1983). However, most hypotheses presented to explain Gregg's phenomenon assume a direct or indirect relation with capillary pressure. The capillary pressure does not depend on perfusion pressure per se, but is influenced by changes in arteriolar resistance. During steady state, autoregulation maintains capillary pressure at a rather constant value. Determination of the dependency of oxygen consumption on capillary pressure (Gregg's phenomenon) is difficult because the rate of change of autoregulation is about equal to the rate of change of oxygen consumption (Dankelman et al., 1989b; Vergroesen and Spaan, 1988). However, the rate of change of regulation can be decreased by a factor of 4 by administration of glibenclamide (Dankelman
et al., 1994). Hence, with glibenclamide, the dynamic response of oxygen consumption following changes in perfusion pressure can be obtained with minor disturbances of changes in arteriolar resistance. In a set of animals, from which we reported the effect of glibenclamide on the rate of resistance (Dankelman et al., 1994), the transients in oxygen consumption were analyzed for the present report.

A capillary pressure hypothesis implies that oxygen consumption is stronger coupled to perfusion rather than to perfusion pressure, since arteriolar resistance may vary because of autoregulation. In previous studies on the dependency of $O_2$ consumption on coronary flow or pressure, variations on coronary flow or pressure were often obtained by infusion of a vasodilatory substance. Vasodilation may induce alterations in microvascular conditions of different kinds. Hence, the dependency of oxygen consumption on coronary pressure or flow measured during vasodilation may be different from the physiological preparation with intact flow control. Moreover, flow alterations induced by pressure alterations at maximal vasodilation are much larger than in the presence of autoregulation. The present approach allows us to determine the importance of Gregg’s phenomenon at normal flow levels by temporarily disturbing the microvascular perfusion. The hypothesis is that oxygen consumption is dependent on coronary perfusion in the presence of flow regulation, but an actual change in oxygen consumption induced by a perfusion pressure step is minimized because of autoregulation.

5.2 Methods

5.2.1 Preparation

The experimental setup is similar to the one described in Chapter 2. Seven goats weighing 19 to 35 kg were treated for worms using ivermectine (MSD) a few weeks prior to the experiment. The goats were anesthetized by intramuscular injection of a mixture of 3 ml ketamine-hydrochloride (Aescoket, 100 mg/ml), 3 ml Rompun (Bayer, FRG, 20 mg/ml) and 4 ml atropine-sulphate (0.5 mg/ml). Anesthesia was maintained by intravenous injection of 30 ml fentanyl (0.05 mg/ml). 2 ml pancuroniumbromide (Pavulon, Organon, 2 mg/ml iv) was given for muscle relaxation. The goats were ventilated with a Harvard respirator using a 2:1 nitrous oxide-oxygen mixture.

A left thoracotomy was performed and the 3rd and the 4th rib were removed. The pericardium was opened and a cradle was formed. The left main coronary artery was dissected and a ligature was placed around it. Another ligature was placed around the great cardiac vein, close to the junction where the
left hemiazygos vein drains into the coronary sinus. The His bundle was destroyed by local injection of formaldehyde (Steiner and Kovalik, 1968) and the right ventricle was paced. A thin catheter was inserted into the left anterior descending vein using the Herd-Barger technique (Herd and Barger, 1964). A catheter tip manometer (PPG Hellige, model HD 36 010 80001, 6F) was inserted through a purse string in the left atrial appendage into the left ventricle. A stainless-steel Gregg cannula was inserted into the aorta via a purse string after administration of 3 ml heparin (5000 IU/ml iv). Without disruption of coronary flow, the cannula was ligated into the left main coronary artery. The great cardiac vein was cannulated via the left hemiazygos vein. Heparin was given by continuous infusion (5000 IU/hour iv). After the control measurements glibencamide (220 ml, 1 mg/ml, 10 ml/min) was given by intracoronary infusion. Glibenclamide was dissolved in a NaOH salt solution with pH=14. HCl was added to obtain the lowest pH without causing precipitation (pH=9). The effect of glibenclamide administration on glucose levels were checked by Haemo-Gliukotest (1-44R, visual color comparison). When needed, glucose levels were restored to normal by infusion (D-Glucose 5%).

5.2.2 Arterial perfusion/venous drainage system

The arterial perfusion system was essentially similar to the one previously described by Spaan et al. (1981, Figure 2.1). Blood from the left carotid artery was pumped into a reservoir via a heat exchanger and filter (Mirospore, 40 μm). A precision pressure regulator (Fairchild, model 10R) held reservoir pressure at a preset value. Perfusion pressure was measured at the cannula tip with a catheter tip manometer (Millar SPC-350, 5F). An electromagnetic cannulating flow probe, interposed in the perfusion line, monitored coronary arterial flow (Statham, 5 mm, model Sp2202). The blood reservoir was equipped with a level controller with feedback to the roller pump, in order to keep the blood level in the reservoir constant.

Blood from the cannulated great cardiac vein drained into a similar pressure-controlled reservoir via a 2.5 mm flow probe (Statham). The pressure in the reservoir was kept constant with a pressure regulator. Venous blood was pumped back into the left jugular vein via a heat exchanger and filter (Mirospore, 40 μm).

Arterio-venous oxygen content difference was continuously measured optically by a technique (Shepherd and Burgar, 1977) modified in our laboratory (Vergroesend and Spaan, 1988).
5.2.3 Measurements and protocol

Left ventricular (P_{lv}), coronary arterial and venous pressure, coronary arterial and venous flow as well as the arterio-venous oxygen content difference were measured and digitized on-line at a sample rate of 80 Hz. pH, arterial P_{o2} and P_{co2} were measured every 30 minutes with an automated blood gas analyzer (model ABL330, Radiometer). Hemoglobin content, arterial and coronary venous oxygen saturations were measured with a hemoximeter (model OSM 2, Radiometer). Several times during the protocol, the perfusion line was clamped for 15 s to check the zero of the arterial flow meter and to measure pressure at zero flow. At the end of the experiment, the weight of the perfused tissue was determined: a mixture of gelatine and white paint (Latex) at 37°C was injected into the left main coronary artery. After cooling in a freezer for approximately half an hour, the colored tissue was dissected and weighed.

The left main coronary artery was perfused with constant pressure perfusion. Stepwise changes in perfusion pressure were made between approximately 80, 100 and 120 mmHg at different heart rates. Before and after every step in perfusion pressure time for stabilization was allowed. The protocol was repeated after administration of glibenclamide.

5.2.4 Data analysis

All signals were digitized on-line for 100 s, starting 15 s before the step change in perfusion. The response of oxygen consumption (M{sub Vo2}) was calculated from beat averaged arterial flow (Q_{a}) and beat averaged arterio-venous oxygen content difference (AVo2) by M{sub Vo2}=Q_{a}AVo2, where AVo2 is compensated for mixing processes in the capillaries and time delay caused by the transport of blood from capillaries to the venous measuring site by:

\[ AVo2^*(t) = \frac{V}{Q_a(t)} \int_{t}^{t+\tau_d} \frac{dAVo2(t+\tau_d)}{dt} + AVo2(t+\tau_d) \]  \hspace{1cm} (5.1)

where:

\( V \) = volume of mixing compartment

\( \tau_d \) = the time delay representing the transport time from the capillaries to the venous measuring site

The AVo2 signal was shifted in time (\( \tau_d \) s) for a full amount of beats which ranged between 2 and 3 s (Ruiter et al., 1978; Van der Ploeg et al., 1993). Furthermore, it was assumed that the oxygen concentration of blood that leaves the capillaries and enters the venules comes from a mixing compartment with
volume V of 10 ml/100 g. This mixing compartment represents the capillaries and tissue (Van der Ploeg et al., 1993).

The coronary resistance index was calculated as the quotient of beat averaged driving pressure (P) and arterial flow (Q_a). Driving pressure was defined as the difference between coronary arterial pressure and wedge pressure. Wedge pressure is the averaged coronary peripheral pressure after 10 s of arterial occlusion. This wedge pressure has been shown to be close to epicardial venous pressure in the goat (Vergroesen, 1990). The resistance index reflects resistance only in steady state or under conditions in which flow and/or pressure vary so slowly that capacitance effects can be ignored.

To compare the time course of the responses to different interventions, MVO_2 and resistance index (P/Q_a) were normalized. For this, these signals were averaged over the first 15 s of control, yielding MVO_2,0 and (P/Q_a),0. The normalized response of MVO_2 and P/Q_a is then given by MVO_2/MVO_2,0 and (P/Q_a)/(P/Q_a),0, respectively.

The t_50 values were defined as the time in seconds after the step in P at which the change in normalized oxygen consumption and resistance index had reached 50% of its final change. To estimate t_50 from the rather noisy signals (e.g. Figure 5.1), a high order polynomial (order 6 to 7) was fitted to a part of the signal using a signal analysis program (Matlab, The MathWorks, Inc). The

![Figure 5.1 Method of calculating t_50 values. A high order polynomial was fitted to the signal starting 2 s after the intervention (a decrease in P). The value of the polynomial at 2 s after the step was used as begin value, while the average of the last 15 s of the signal was used as end value. t_50 was defined as the time after the start of intervention at which the polynomial reached 50% of the difference between begin and end value.](image_url)
Figure 5.2 Typical recordings showing the response to a stepwise decrease in perfusion pressure during control (left panels) and after administration of glibenclamide (right panels). $P_{LV}$: left ventricular pressure, $P_p$: perfusion pressure, $Q_a$: coronary arterial flow, $AVO_2$: arterio-venous oxygen content difference.

first 2 s after the step were not included in the polynomial fitting because during this period changes in flow are strongly influenced by capacitance effects (Dankelman et al., 1989a).

To establish the average time course of the normalized responses to the several interventions, the responses of each animal were grouped according to intervention and averaged. These curves averaged per animal were then grouped again according to intervention and averaged over all animals. In the averaging procedure, each beat averaged function of time was assumed to be staircase-like: constant during a heart beat and changing abruptly at the beginning of systole of the next heart beat. Hence, the resistance index is defined at each moment of digital sampling, enabling average responses to be calculated.

Statistical significance of differences was tested using the two-tailed paired t-test. $P<0.05$ was assumed to be significant.

5.3 Results

Typical phasic tracings obtained by increasing perfusion pressure are depicted in Figure 5.2 for control (left panels) and glibenclamide conditions (right
Figure 5.3 Signals from the same intervention as in Figure 5.2, averaged per beat.
The responses of $Q_a$, $P/Q_a$ and $Q_a\cdot AVO_2$ are slowed down after administration of glibenclamide.

panels). Flow increased with pressure and subsequently decreased as result of vasoconstriction. With glibenclamide this decrease was slower than during control. The bottom panels provide the responses of arterio-venous oxygen content difference. In both conditions $AVO_2$ changes after a delay of a few seconds. This time delay is caused by the transport of blood from capillaries to the venous measuring site. Note that with glibenclamide the $AVO_2$ changes at a slower rate than during control. In Figure 5.3 perfusion pressure, flow and $AVO_2$ from the same intervention as shown in Figure 5.2 are averaged per beat. Typical responses of $P/Q_a$ and $Q_a\cdot AVO_2$ are added. These responses clearly show a decreased rate of change after glibenclamide administration.

The average responses of $MV O_2$ ($Q_a\cdot AVO_2^*$) resulting from decreasing and increasing $P_p$ are depicted in Figure 5.4. The effect of the correction procedure for the calculation of $MV O_2$ may become clear from the uncorrected $Q_a\cdot AVO_2$ signal. Note that in all cases oxygen consumption changes due to the pressure step and thereafter returns to its initial level. Administration of glibenclamide decreases the rate of return.

The initial mean values of perfusion pressure, coronary arterial flow, systolic left ventricular pressure, maximal $dP/L/dt$ and oxygen consumption before and after administration of glibenclamide are provided in Table 5.1. The systolic
Table 5.1 Hemodynamic data.

<table>
<thead>
<tr>
<th></th>
<th>(P_p)</th>
<th>(Q_a)</th>
<th>(M_{Vo_2})</th>
<th>(P_{IV,Syst})</th>
<th>(P_{IV,Syst,5})</th>
<th>(dP_{IV}/dt_{max})</th>
<th>(dP_{IV}/dt_{max,5})</th>
<th>(n)</th>
<th>(n_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control up</td>
<td>96±3</td>
<td>1.6±0.2</td>
<td>95±10</td>
<td>90±5</td>
<td>92±5</td>
<td>1709±196</td>
<td>1755±212</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>glib up</td>
<td>95±4*</td>
<td>1.4±0.2**</td>
<td>78±8**</td>
<td>97±6*</td>
<td>97±6*</td>
<td>1540±219*</td>
<td>1520±224*</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>control down</td>
<td>114±5</td>
<td>1.8±0.2</td>
<td>100±12</td>
<td>85±6</td>
<td>86±6</td>
<td>1661±223</td>
<td>1662±225</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>glib down</td>
<td>116±4*</td>
<td>1.7±0.2**</td>
<td>86±10*</td>
<td>94±8*</td>
<td>94±8*</td>
<td>1629±254*</td>
<td>1759±360*</td>
<td>7</td>
<td>26</td>
</tr>
</tbody>
</table>

Steady state values before the pressure step during control and after glibenclamide (glib) administration. \(P_{IV,Syst}\) and \(dP_{IV}/dt_{max}\) are the values averaged over the first 5 seconds following a step in perfusion pressure. Values are means ± SE, means and SE are calculated from means per animal, \(n\): number of animals, \(n_i\): total numbers of interventions. Control values are compared with values after administration of glibenclamide using the paired t-test. Differences between \(P_{IV,Syst}\) and \(P_{IV,Syst,5}\) as well as \(dP_{IV}/dt_{max}\) and \(dP_{IV}/dt_{max,5}\) were not significant. *: P<0.01, **: P<0.001, *: not significant.

Oxygen Consumption

**Figure 5.4** Summary of the normalized oxygen consumption response as a result of changes in perfusion pressure (n=7 animals) during control (left panels) or after glibenclamide administration (right panels). Upper panels: results from a decrease in perfusion pressure. Lower panels: results from an increase in perfusion pressure. Solid line: \(M_{Vo_2}\) (\(Q_a\cdot A_{Vo_2}\)), broken line: \(Q_a\cdot A_{Vo_2}\) (not corrected).

left ventricular pressure and maximal \(dP_{IV}/dt\) averaged over the first 5 seconds following the step in perfusion pressure are given as well. Note that although \(M_{Vo_2}\) changed in the first 5 seconds after the pressure step (Figure 5.4), systolic left ventricular pressure and maximal \(dP_{IV}/dt\) did not change.
Figure 5.5 Summary of the normalized resistance index (P/Qa) responses as a result of changes in perfusion pressure (n=7 animals) during control (left panels) or after glibenclamide administration (right panels). Upper panels: results from a decrease in perfusion pressure. Lower panels: results from an increase in perfusion pressure.

The average responses of P/Qa are depicted in Figure 5.5. These responses have an initial reversed phase. An increase in perfusion pressure induces a sudden decrease in resistance (P/Qa) due to mechanical effects of distention (Dankelman et al., 1989b). From this event on, the response is dominated by changes in smooth muscle tone and reflects the dynamics of coronary regulation (Dankelman et al., 1990). Due to glibenclamide, transients in coronary resistance caused by regulation become slower compared with control (Dankelman et al., 1994).

The t_{50} values for the rate of return of MVo2 and P/Qa are compared in Figure 5.6. Again it is demonstrated that the rate of change of both signals is reduced by administration of glibenclamide. Note further that the rate of change of MVo2 and P/Qa are in the same range for each condition: i.e. with and without glibenclamide. Without glibenclamide there is no difference in t_{50} between MVo2 and resistance. However, after glibenclamide administration the t_{50} value for the MVo2 change is smaller than the t_{50} for the resistance change.

The maximal change in MVo2 response during transients as well as the steady state change in MVo2 are given in Figure 5.7. The maximal change in Qa·AVo2 (not compensated) is given as well, showing the effect of correction of the AVo2 signal. After glibenclamide administration, the maximal change in oxygen consumption during transients is larger than the change in the control
Figure 5.6 Bar diagram summarizing t_{50} values (means±SE) for Q_{a}·AVO_{2}· and P/Q_{a} following P_{p} steps (n=7 animals). t_{50} values are higher after administration of glibenclamide. There is a difference in t_{50} values between the dilation and constriction response only after administration of glibenclamide. The levels of significance are *: P<0.05, **: P<0.001.

Figure 5.7 Bar diagram summarizing the relative maximal change in oxygen consumption immediately after the step in perfusion pressure and the steady state change (means±SE). Gray bars: maximal change in Q_{a}·AVO_{2}, middle bars: maximal change in MVO_{2} (Q_{a}·AVO_{2}·), right bars: steady state change (end values). Upper panels: results after an increase in P_{p}, lower panels: results after a decrease in panels. Left panels: control, right panels: after glibenclamide administration.
situation. Moreover, during control and after glibenclamide administration the maximal changes in MVo$_2$ transients were significantly higher than the steady state changes. The steady state level of oxygen consumption (steady state Gregg’s phenomenon) was influenced by perfusion pressure only during control. With glibenclamide this steady state effect was not measurable (Figure 5.7, end values).

5.4 Discussion

In this study the transient responses of oxygen consumption following changes in perfusion pressure are compared with transient responses of coronary resistance. The effect of a decrease in rate of regulation by the administration of glibenclamide (Dankelman et al., 1994) on the transients in oxygen consumption was analyzed. The results indicate that transients in oxygen consumption depend on the rate of change of autoregulation. Furthermore, during transients large variations in oxygen consumption can occur when the rate of regulation is slow.

5.4.1 Methodological limitations

A key problem for the present study is the limitation of deducing a changing oxygen consumption from the time varying signals of coronary arterial flow and arterio-venous oxygen content difference. The oxygen uptake of the heart, calculated from the product of coronary flow and measured arterio-venous oxygen content difference of blood, only equals myocardial oxygen consumption under steady state conditions (Ruiter et al., 1978). Under dynamic conditions, some delay between the actual myocardial O$_2$ consumption and O$_2$ uptake of the heart occurs. For a part, this delay is caused by the transport of blood from the site of oxygen consumption to the venous measuring site. However, the rapid O$_2$ diffusion and the many capillary and venous anastomoses cause mixture of blood with different O$_2$ saturation. Hence, the difference between the actual O$_2$ consumption and the O$_2$ uptake calculated from the product of flow and measured AVo$_2$ cannot be approximated by a simple time delay, but is more complex. The dynamics of O$_2$ mixing in the O$_2$ stores within the tissue, which are formed by the hemoglobin in blood, the myoglobin in tissue and the physically dissolved oxygen, depend on the ratio of O$_2$ storage and O$_2$ supply (Chapter 2). Hence, these dynamics depend on e.g. vessel volume and coronary flow. As an approximation, one could assume a fixed dynamic behavior for the O$_2$ mixing process. Then, the difference between the actual O$_2$ consumption and O$_2$ uptake calculated from flow times AVo$_2$ will be smaller after glibenclamide administration, since glibenclamide decreases the rate of flow adaptation and
hence the rate of flow-induced changes in blood and tissue Po2.

The translation between measured oxygen uptake and oxygen consumption was made by correcting the measured AVo2(t) with a simple O2 exchange model consisting of two compartments (Equation 5.1, Figure 2.2). The first compartment represents the oxygen exchange compartment and is assumed to be well mixed. The response of the oxygen content of the outflow of this compartment to either a step change in flow or in oxygen consumption is exponential with a time constant (τmix) determined by the compartment volume (V) and coronary flow (Qs): τmix = V/Qs. Blood from the first compartment is transported to the venous measuring site through the second compartment. Since this compartment is assumed to be unmixed, it causes a time delay τd of the venous O2 content. The magnitudes of the time constant and time delay determine the difference between the corrected and uncorrected signals. It should be noted that the volume V and time delay were taken constant for all experiments. The values are based on earlier studies (Ruiter et al., 1978; Van der Ploeg et al., 1993).

One may wonder to what extent the calculated MVO2 (Qa·AVo2·) indeed represents the transients in oxygen consumption induced by a step change in pressure. If the applied correction is perfect, the results would imply that oxygen consumption changes instantly after a pressure step (Figure 5.4). This is unlikely in the light of reported time constants for changes in oxygen consumption which are in the order of 6 s (Van Beek and Westerhof, 1991; Vergroesen and Spaan, 1988). It is more likely that our model assumptions are oversimplified. However, an upper bound for the accuracy in obtaining transient information can be deduced from the measurements during control. If one assumes that, during control, resistance changes are fast enough to prevent an O2 consumption response due to the change in perfusion, the overshoot in the predicted oxygen consumption would be completely artificial. The normalized O2 consumption response is constant at about 5 s after a perfusion pressure increase (Figure 5.4). Therefore, one may assume that our method predicts oxygen consumption correctly within about 5 s. For two reasons this time must be considered as an upper bound for the case with glibenclamide. First, with a time constant of 6 s for the rate of oxygen consumption changes one may expect some deflections as observed. Second, the rate of resistance change after glibenclamide administration is 3 to 5 times slower, reducing the effect of fast changes in the oxygen storage. Based on this reasoning, one may safely state that, after glibenclamide administration, the oxygen consumption at 5 s after the pressure step is well predicted by our corrected signal.

We have reported the peak values for oxygen consumption (Figure 5.7). In the condition with glibenclamide the maximal oxygen consumption change was 20-30%, after a pressure change of 20 mmHg. When our peak value is an overestimate of the maximal change in oxygen consumption, the oxygen
consumption at 5 s after the pressure step could be taken as the maximal response. This increase in oxygen consumption is still 90% of the peak increase. However, at that moment vasomotor tone has corrected resistance by a similar amount. Assuming that the time course of resistance reflects the time course of the capillary pressure change, the stimulus for the increased oxygen consumption at 5 s after the pressure step has changed by the same amount as oxygen consumption. Hence, the sensitivity of oxygen consumption to a change of perfusion seems to be well represented by the numbers on maximal deflections for glibenclamide.

The effect of the vehicle in the glibenclamide protocol was reported in our earlier study (Dankelman et al., 1994). No effect of vehicle on the rate of change of regulation was found, whereas subsequent administration of glibenclamide decreased the rate of regulation.

5.4.2 Dynamics of Gregg’s phenomenon

In earlier studies, it was reported that after a coronary occlusion not only a flow repayment, but also an oxygen repayment would occur (e.g. Coffman and Gregg, 1961). It was discussed by Ruiter et al. (1978) that the reported O₂ repayment ratios were overestimated because of a mismatch between the coronary flow signal and the measurement of the arterio-venous oxygen content difference. Although the authors could not quantify the actual repayment very accurately, they doubted whether a ratio higher than 1 is likely. The present study points to two complications for the interpretation of reactive hyperemia in terms of oxygen debt and oxygen repayment. Since perfusion pressure is strongly reduced during occlusion, oxygen consumption due to Gregg’s phenomenon will be decreased and hence the oxygen debt built up during the occlusion will be smaller than simply the product of oxygen consumption during control and the duration of occlusion. Second, in the period of reactive hyperemia oxygen consumption may be above control because of restoration of perfusion pressure and vasodilation.

Especially in earlier studies on coronary flow control, a direct coupling between flow and oxygen consumption was supposed (Eckenhoff et al., 1947). If such a direct coupling would exist, one may wonder why flow would not remain at a higher level when oxygen consumption is increased by the increased pressure during glibenclamide. As is clear from the present study, both flow and oxygen consumption are regulated back to their control values and therefore it is most likely that the change in resistance precedes the change in oxygen consumption. The fact that the perfusion pressure induced increase in oxygen consumption is not maintained implies that the factor responsible for the coupling between tissue parameters and coronary resistance is more affected by the increased flow than by the increased metabolism. This point of view may be
elucidated by the oxygen control model presented in earlier studies on the
dynamics of coronary flow control (Dankelman et al., 1989a; Dankelman et al.,
1990). In this model it is assumed that tissue \( \text{PO}_2 \) is coupled to coronary
resistance by an unknown factor. In our present study a 20% increase in
perfusion pressure induced about 40% increase in flow and a 30% increase in
oxygen consumption. Thus, the increase in flow is initially larger than the oxygen
consumption increase, leading to an increase in tissue \( \text{PO}_2 \), which in the model
is a stimulus for vasoconstriction.

5.4.3 Gregg's phenomenon and heart function

In our study the oxygen consumption increase coupled to perfusion
(Gregg's phenomenon) was not accompanied by improvement of cardiac function
in so far parameters could be estimated. No statistically significant correlation
between the maximal increased oxygen consumption and systolic left ventricular
pressure or maximal \( \text{dP} \text{/dt} \) was found. Hence, the increased oxygen consumption
must be regarded as energy loss. This energy might have been used for internal
compensation by the myocytes for changes in mechanical properties of the
myocardium. It must be noted that some studies do report an increase in
mechanical work induced by the increased perfusion (Arnold et al., 1968;
Kitakaze and Marban, 1989; Schouten et al., 1992). However, there may be
differences between the experimental preparations, e.g. isolated hearts or papillary
muscles versus intact \textit{in situ} beating heart.

Since due to Gregg's phenomenon oxygen consumption changed after a
perfusion increase, one must conclude that under normal conditions part of the
oxygen consumption of the heart is related to the determinant of Gregg's
phenomenon, which probably is capillary pressure. However, from the present
study it is difficult to estimate the amount of perfusion induced \( \text{MVCO}_2 \) in steady
state. Since a 20% change in pressure resulted in a 20% change of oxygen
consumption, linear extrapolation to zero pressure would imply that the total
oxygen consumption would be related to Gregg's phenomenon. Therefore, it is
most likely that the process is non-linear. Non-linearity is suggested by a
significantly lower undershoot in \( \text{MVCO}_2 \) by a pressure decrease compared to the
overshoot in \( \text{MVCO}_2 \) by a pressure increase.

Based on the conclusion that the efficiency of oxygen usage in the heart
is lowered by Gregg's phenomenon and on the many experimental results
suggesting that capillary pressure is the determinant of Gregg's phenomenon, it
is plausible that a situation of high arterial vasoconstriction is strived for via
coronary regulation, since this results in a minimized capillary pressure and
thereby minimal energy loss.
6 Effect of heart contraction on the functional distribution of coronary volume in goats

In eight anesthetized open chest goats the His bundle was destroyed and the hearts were alternately paced at 60, 90 and 130 beats per minute. The left main coronary artery was cannulated and perfused at constant flow. The response of the coronary arterio-venous oxygen content difference \((\text{AVo}_2)\) to a flow step was measured by determination of the color difference of arterial and venous blood. By comparison of this transient with simulations from a coronary \(O_2\) exchange model, the volume of the vessels participating in \(O_2\) exchange \((V_m)\) and the volume of the venous transport vessels \((V_{\text{ven}})\) were estimated at the different heart rates (HR).

During control an effect of HR on coronary volume could not be established: mean volumes \(\pm\) SE at HR 60, 90 and 130 were respectively \(V_m = 9.5\pm0.8\) (n=6), 8.6\pm1.6 (n=4), 9.7\pm0.8 (n=7) and \(V_{\text{ven}} = 2.3\pm0.4, 3.2\pm0.5\) and 2.7\pm0.5 ml/100 g. Maximal vasodilation with adenosine resulted in an increase in \(V_m\) when HR increased. For HR 60, 90 and 130 we found \(V_m = 11.0\pm1.7\) (n=7), 14.6\pm2.3 (n=5), 24.0\pm3.6 (n=8) and \(V_{\text{ven}} = 3.7\pm0.4, 4.4\pm0.3\) and 3.3\pm0.4 ml/100 g. Especially during vasodilation these results depended largely on the assumed time course of myocardial oxygen consumption \((\text{MVO}_2)\). Since this time course cannot be measured, the results are less reliable in those cases where the change in \(\text{MVO}_2\) is large.

Selection of the flow steps with a relatively small contribution of the \(\text{MVO}_2\) change to the \(\text{AVo}_2\) transient resulted in a smaller dependency of the \(V_m\) estimations on the assumed \(\text{MVO}_2\) time course. Although the increase in \(V_m\) with HR during vasodilation became smaller, a discrepancy between these results and measurements of the total coronary volume change after a heart rate step by integration of the difference between arterial and venous flow remained.

Although model assumptions deviating from the concept of a well-mixed oxygen exchange compartment have been analyzed, we have not been able to come up with a satisfactory explanation why at high heart rate blood volume in the exchange compartment would be higher than at low heart rate, and only during vasodilation. This indicates the limits of our present concepts on oxygen exchange and distribution in the heart.

6.1 Introduction

Knowledge of coronary capillary blood volume is relevant for understanding different mechanisms, varying from oxygen exchange to the effect of cardiac contraction on intramural vessels (Spann, 1991). Capillary blood volume is not constant but depends on e.g. the level of perfusion, heart rate and venous pressure (Morgenstern et al., 1973; Ziegler and Goeresky, 1971; Judd et al., 1993; Salisbury et al., 1961; Judd and Levy, 1991). The effects of these
determinants on coronary volume are experimentally quantified, but information on a distributed effect, i.e. on capillary versus venous volume, is hardly available. In the present study it is attempted to establish the effect of heart rate on coronary capillary and coronary venous volume using hemoglobin-bound oxygen as an endogenous tracer.

The method used to estimate the coronary volume in \textit{in situ} beating hearts has been presented in Chapter 2. With this method, the response of the arteriovenous oxygen content difference (AVO$_2$) after a step in coronary arterial flow is interpreted by an oxygen exchange model for the coronary circulation consisting of two compartments: 1. the vessels participating in oxygen exchange, which are assumed to be well mixed, 2. the vessels distal of these, which are assumed to be unmixed. Through comparison of simulations from this model and measured signals, the volumes of the two compartments are estimated. Hence, a coronary volume distribution according to O$_2$ exchange properties can be made under physiological conditions. However, the model simulations require a quantification of myocardial O$_2$ consumption (MVO$_2$) at each moment. Although MVO$_2$ can be calculated under static conditions by multiplication of coronary flow and AVO$_2$, the time course of changes in MVO$_2$ can hardly be determined from measurable parameters. This is a drawback for our method of volume estimation, since a change in flow induces a change in oxygen consumption (Gregg’s phenomenon) (Gregg, 1963; Abel and Reis, 1970; Zborowska-Sluis, 1977). Hence, assumptions on the MVO$_2$ transients must be made to estimate the coronary volume distribution. These assumptions will cause an uncertainty in the volume estimations, which obviously is larger when the change in MVO$_2$ is larger.

In this study the AVO$_2$ response to a flow step at different heart rates (HR) was analyzed, and it was attempted to interpret these signals in terms of volume distribution between capillary and venous volume. Measurements were made in open chest anesthetized goats under control circumstances and during maximal vasodilation. The effect of changes in the myocardial oxygen consumption on the volume estimations was assessed.

### 6.2 Methods

#### 6.2.1 Estimation of coronary volume participating in O$_2$ exchange and distal coronary venous volume

The method to estimate the coronary volume distribution is introduced in Section 2.2.5 and partly repeated and extended in this section. Calculations are made with signals that were averaged per heart beat. The measured time response
of the arterio-venous oxygen content difference (AVo₂) to a flow step is compared with model predictions. The model is a simplified representation of the coronary bed and consists of a well-mixed compartment, from which oxygen is consumed, and a distal unmixed compartment without oxygen consumption (Figure 2.2). The well-mixed compartment represents the vessels and surrounding tissue responsible for oxygen exchange, whereas the unmixed compartment represents the venous vessels. The volumes of the two compartments, which are assumed not to be affected by the intervention, are the parameters to be estimated.

Because of the oxygen mass balance, the difference between in- and outflow of oxygen must equal the sum of oxygen consumption and change in oxygen stored. For the mixed compartment, this can be written as follows:

\[
\frac{dA(t)}{dt} = Q_a(t) \cdot [O_2]_a(t) - Q_o(t) \cdot [O_2]_o(t) - \text{MVo}_2(t) \tag{6.1}
\]

where:
A(t) = total amount of O₂ in blood and tissue in the mixed compartment [µl O₂/100 g]
Q_a(t) = arterial flow [ml/s/100 g]
[O₂]_a(t) = arterial O₂ content [µl O₂/ml blood]
[O₂]_m(t) = O₂ content of blood in the mixed compartment [µl O₂/ml blood]
MVo₂(t) = myocardial O₂ consumption [µl O₂/s/100 g]
t = time

Oxygen consumption is written as a function of time since it may change during the response to the flow step as described by Gregg (1963).

The amount of oxygen in this compartment at time t, A(t), is the sum of O₂ in blood and in tissue:

\[
A(t) = V_m \cdot [O_2]_m(t) + V_t \cdot [O_2]_t(t) \tag{6.2}
\]

where:
V_m = vessel volume in the mixed compartment [ml/100 g]
V_t = volume of tissue in the mixed compartment [ml/100 g]
[O₂]_t(t) = O₂ content in tissue [µl O₂/ml tissue]

In the blood, only the oxygen bound to hemoglobin was considered, since the amount of oxygen dissolved is negligible compared to bound O₂. Hence, the blood oxygen content depends on the oxygen binding capacity of hemoglobin and hemoglobin saturation and concentration, whereas the tissue oxygen content is
dependent on the oxygen binding capacity, amount and saturation of myoglobin and on the amount of oxygen dissolved in the tissue. This is expressed in the following equation:

\[ A(t) = V_m \cdot 1.36 \cdot 10 \cdot \text{Hb} \cdot S_{\text{Hb,m}}(t) + V_t \cdot (1.36 \cdot 10 \cdot \text{Mb} \cdot S_{\text{Mb}}(t) + \sigma \cdot \text{Po}_{2,t}(t)) \]  

where:
- \( S_{\text{Hb,m}}(t) \) = hemoglobin saturation of blood in the mixed compartment [-]
- \( S_{\text{Mb}}(t) \) = myoglobin saturation [-]
- 1.36 = \( O_2 \) binding capacity of hemoglobin or myoglobin [ml \( O_2 \)/g] (Schmidt and Thews, 1989)
- \( \text{Hb} \) = hemoglobin content [g/100 ml blood]
- \( \text{Mb} \) = myoglobin content (0.4 g/100 ml tissue, Schmidt and Thews, 1989)
- \( \sigma \) = \( O_2 \) solubility of extravascular tissue (0.028 \( \mu l \) \( O_2 \)/ml tissue/mmHg, Altman and Dittmer, 1970)
- 10 = factor to arrive at the dimension \( \mu l \) \( O_2 \)/ml
- \( \text{Po}_{2,t} \) = partial oxygen pressure in the tissue [mmHg]

Because the volumes are assumed to be constant during one intervention, the change in the amount of oxygen in the mixed compartment can be written as:

\[ \frac{dA(t)}{dt} = V_m \cdot \frac{d(1.36 \cdot 10 \cdot \text{Hb} \cdot S_{\text{Hb,m}}(t))}{dt} + V_t \cdot \frac{d(1.36 \cdot 10 \cdot \text{Mb} \cdot S_{\text{Mb}}(t) + \sigma \cdot \text{Po}_{2,t}(t))}{dt} \]  

The changes of \( \text{Hb} \) and \( \text{Mb} \) saturation and \( \text{Po}_2 \) can be expressed in a change of the measured variable, i.e. venous oxygen content \([O_2]_v(t)\). The changes in hemoglobin and myoglobin saturation are related to changes in oxygen pressure according to the respective saturation curves. These curves are non-linear but may be linearized around a working point defined by the average \( \text{Po}_2 \). Rewriting of Equation 6.4 and taking into account Equations 6.2 and 6.3 results in:

\[ \frac{dA(t)}{dt} = V_m \cdot 1.36 \cdot 10 \cdot \text{Hb} \cdot \frac{d\lambda_{\text{Hb,m}}}{dt} \cdot \left( \frac{d\text{Po}_{2,m}(t)}{dt} \right) + V_t \cdot (1.36 \cdot 10 \cdot \text{Mb} \cdot \frac{d\lambda_{\text{Mb}}}{dt} + \sigma \cdot \frac{d\text{Po}_{2,t}(t)}{dt}) \cdot \left( \frac{d[O_2]_v(t)}{dt} \right) \]  

\[ = \left( \frac{V_m}{\lambda_{\text{Hb,m}}} \cdot \frac{d\text{Po}_{2,m}(t)}{dt} \right) + \left( \frac{V_t}{\lambda_{\text{Mb}}} \cdot \frac{d\text{Po}_{2,t}(t)}{dt} \right) \cdot \left( \frac{1.36 \cdot 10 \cdot \text{Hb} \cdot \frac{d\lambda_{\text{Hb,v}}}{dt} \cdot \frac{d[O_2]_v(t)}{dt}}{} \right) \]  

\[ \text{Equation 6.5} \]
where:
\[ P_{O_2,m} = \text{partial oxygen pressure of blood in the mixed compartment} \]
\[ P_{O_2,v} = \text{partial oxygen pressure of blood leaving the mixed compartment} \]
\[ \lambda_{Hb,m} = \text{slope of } P_{O_2}\text{-hemoglobin saturation curve linearized at } P_{O_2,m} \text{ [mmHg}^{-1}] \]
\[ \lambda_{Hb,v} = \text{slope of } P_{O_2}\text{-hemoglobin saturation curve linearized at } P_{O_2,v} \text{ [mmHg}^{-1}] \]
\[ \lambda_{Mb} = \text{slope of linearized } P_{O_2}\text{ myoglobin saturation curve [mmHg}^{-1}] \]

In essence, in a mixed compartment the partial oxygen pressures in the capillaries and tissue are equal to the \( P_{O_2} \) of the blood leaving the compartment. Hence, Equation 6.5 can be simplified to:

\[
\frac{dA(t)}{dt} = \left( V_m + V_t \cdot \frac{1.36 \cdot 10 \cdot Mb \cdot \lambda_{Mb} \cdot \sigma_t}{1.36 \cdot 10 \cdot Hb \cdot \lambda_{Hb}} \right) \cdot \frac{d[O_2]_m(t)}{dt} \tag{6.6}
\]

Combining Equations 6.1 and 6.6 yields the following mass balance equation for the mixed compartment:

\[
(V_m + C) \cdot \frac{d[O_2]_m(t)}{dt} = Q_a(t) [O_2]_a(t) - Q_a(t) [O_2]_m(t) - MV_O_2(t) \tag{6.7}
\]

where the correction term \( C \) in ml/100 g for the change in tissue \( O_2 \) storage is:

\[
C = V_t \cdot \frac{\lambda_{Mb} \cdot 1.36 \cdot 10 \cdot Mb + \sigma_t}{\lambda_{Hb} \cdot 1.36 \cdot 10 \cdot Hb} \tag{6.8}
\]

When \( O_2 \) consumption is constant, Equation 6.7 is a first-order differential equation. In this case, the time response of \([O_2]_m\) to a step in flow will be exponential and can be characterized by a time constant \( \tau = (V_m + C)/Q_{a,2} \), where \( Q_{a,2} \) is the arterial flow after the flow step.

The second compartment only causes a time delay \( (\tau_d) \) in appearance of changes of the venous \( O_2 \) content dependent on its volume \( V_{u,m} \) and flow:

\[
\tau_d = \frac{V_{u,m}}{Q_{a,2}} \tag{6.9}
\]
\([O_2]_a\) is practically constant. Hence, \(\text{AVo}_2(t)\) and \([O_2]_m\) are related:

\[
\text{AVo}_2(t) = [O_2]_a - [O_2]_m(t-\tau_d)
\]

Combining Equations 6.7 and 6.10 yields:

\[
-(V_m+C)\cdot \frac{d\text{AVo}_2(t+\tau_d)}{dt} = Q_a(t)\cdot\text{AVo}_2(t+\tau_d) - \text{MVo}_2(t)
\]

Equations 6.9 and 6.11 together provide the dynamic mass balance of the two compartmental model. The parameters \(V_m\) and \(V_{um}\) can be estimated by fitting the solutions of the equations to measured responses of \(\text{AVo}_2(t)\). However, such a procedure requires knowledge of \(\text{MVo}_2(t)\).

From Equation 6.11 it is clear that changes in both flow and oxygen consumption will cause a change in \(\text{AVo}_2\). The method was designed to estimate \(V_m\) from \(\text{AVo}_2(t)\) in response to a flow step under the assumption that \(\text{MVo}_2(t)\) is constant. However, the flow change also affected the myocardial oxygen consumption: \(\text{MVo}_2\) changed in the same direction as flow. Although arterial flow is known at each moment, oxygen consumption can only be calculated accurately in steady state. Therefore assumptions on its time course were necessary. An exponential change with a time constant of 6 seconds was assumed. This time constant was intermediate of the values from two studies in which heart rate was changed. Vergroesen and Spaan (1988) measured a 50% change of \(O_2\) consumption after cardiac arrest in 3.8 s. Assuming an exponential time course, this corresponds to a time constant of 5.5 s. Van Beek and Westerhof (1991) found a mean response time of cardiac mitochondrial oxygen consumption after a heart rate change of 7.7 s in isolated, Tyrode-perfused rabbit hearts at 37 °C. To investigate the effect of the assumed transient of oxygen consumption on the estimations of coronary volume, calculations were also made with time constants of 0, 3 and 9 s.

The assumption of an oxygen consumption time course introduces uncertainty in the simulation of the \(\text{AVo}_2\) response with the model. Differences in the assumed and actual \(\text{MVo}_2\) time course will affect the calculated \(\text{AVo}_2\) transient more when the change in \(\text{MVo}_2\) is larger. Hence, the volume estimations will probably be more reliably when the change in myocardial oxygen consumption is smaller. To be able to quantify this line of reasoning, the model equation for the mixed compartment was linearized. The unmixed compartment does not affect the shape of the \(\text{AVo}_2\) response. Therefore, this compartment is not included in this analysis of the effect of \(\text{MVo}_2\) changes on the volume estimations. Since the volume of the mixed compartment is assumed to be
constant during an intervention, linearization of Equation 6.11 results in:

\[-(V_m+C) \cdot \frac{d(\Delta AV_o_2(t))}{dt} = \Delta Q_a(t) \cdot AV_o_2,0 + Q_a,0 \cdot \Delta AV_o_2(t) - \Delta MVo_2(t) \]  \(\{6.12\}\)

where:
\(AV_o_2,0\) = the arterio-venous \(O_2\) content difference in the working point
\(Q_a,0\) = the arterial flow in the working point
\(\Delta\) = the deviation of the signal from the working point

For steady conditions this equation can be rewritten:

\[\Delta AV_o_2 = - \frac{AV_o_2,0}{Q_a,0} \cdot \Delta Q_a + \frac{1}{Q_a,0} \cdot \Delta MVo_2\]  \(\{6.13\}\)

From this equation it can be seen that the change in \(AV_o_2\) caused by the flow step is in the opposite direction of the \(AV_o_2\) change caused by the \(MVo_2\) change. Furthermore, the final change in \(AV_o_2\) after a flow step will consist of a contribution by the flow change (\(C_{Q_a}\)) and a contribution by the change in \(MVo_2\) (\(C_{MVo_2}\)):

\[AV_o_2,2 - AV_o_2,1 = - \frac{AV_o_2,0}{Q_a,0} \cdot (Q_{a,2} - Q_{a,1}) + \frac{1}{Q_a,0} \cdot (MVo_2,2 - MVo_2,1)\]  \(\{6.14\}\)

where:
subscript \(,1\) = steady state value before the flow step
subscript \(,2\) = steady state value after the flow step

Errors in the flow, resulting from measurement, but especially errors in oxygen consumption, resulting from a deviation between actual and assumed time course, will affect the \(AV_o_2\) transient more when the relative contribution is larger. In other words, if oxygen consumption remains almost constant, an error in the assumption of the \(MVo_2\) time course is not relevant. However, if the contribution of the \(MVo_2\) change to the \(AV_o_2\) transient becomes large compared to the flow effect, an error in the assumed \(MVo_2\) time course will be dominant. To investigate the possible error on the volume estimation introduced by the \(MVo_2\) change, the \(C_{MVo_2}/C_{Q_a}\) ratio was calculated for each intervention.

As described in Section 2.2.5, simulations from the \(O_2\) exchange model were fitted to the experimental arterio-venous \(O_2\) content difference by varying
time delay and mixed volume and by using a least-squares method. Measured arterial flow and an assumed O₂ consumption time course were used as input to the model. The moments at which the measured AVo₂(t) had changed relatively 10 and 90% determined the time period for which the sum of squared differences between measured AVo₂(t) and model simulations was minimized. The volume of the O₂ exchange vessels was obtained by correcting the estimated mixed compartment volume for the change in tissue oxygen storage (Equation 6.8), and the volume of the venous transport vessels was calculated by multiplication of the estimated delay time with the flow after the flow step (Equation 6.9).

From the difference between arterial inflow and venous outflow, changes in total coronary volume after flow and heart rate steps were calculated. This method is described in Section 2.2.6.

6.2.2 Preparation, perfusion system and protocol

Data from eight goats were used in this study. Data from six of these animals were also used in Chapter 4. A complete description of the preparation and the arterial perfusion/venous drainage system is given in Section 4.2. In short, in anesthetized, open-chest goats the His bundle was destroyed by local injection of formaldehyde (37%) and the hearts were paced. The left main coronary artery and great cardiac vein were cannulated and connected to a perfusion/drainage system. Left ventricular, coronary arterial and venous pressures, as well as coronary arterial and venous flows and AVo₂ were sampled at 80 Hz.

The protocol started with a 15 s occlusion of the arterial perfusion line and a 2 s occlusion of the venous drainage line to check for the zeros of the flow probes. This was repeated several times during the protocol. Between the occlusions, the perfusion condition was changed from constant pressure perfusion to constant flow perfusion by placing a resistance on the perfusion line distal to the pressure reservoir and raising the pressure in the reservoir to compensate for the increased resistance. With the heart rate (HR) alternating between ~60 and ~130 beats/min, flow steps were induced by stepwise changing the reservoir pressure between two levels. In 3 animals, additional flow steps were performed at a heart rate of 90 beats/min. Hence, data at HR 60 and 130 were obtained in comparable conditions, whereas the data at HR 90 may be influenced by a changed condition of the preparation. Before every flow step, venous blood samples were taken and time for stabilization of pressure and AVo₂ signals was allowed. After these control measurements, which are referred to as "control" throughout this chapter, adenosine was administered to obtain maximal
Effect of Heart Rate on Coronary Volume

vasodilation. To test for maximal dilation, the arterial perfusion line was clamped for 15 s during constant pressure perfusion. If reactive hyperemia had not yet disappeared completely, the intracoronary infusion rate of adenosine (5 mM) was increased. The complete protocol was repeated at maximal vasodilation.

In two goats the protocol was slightly different. In one animal, only data during maximal vasodilation were obtained. In the other animal, a complete heart block could not be made. Flow steps were made at heart rates alternating between 80 and 175 beats/min, with additional flow steps at a heart rate of 130 beats/min. The data at HR 80 were grouped with the data at HR 90 from other animals, whereas the data at HR 175, which were obtained in just one animal, are only presented in Figure 6.3.

6.2.3 Statistics

Data obtained during control and maximal vasodilation were grouped according to heart rate and averaged per animal. Reported data are means ± SE of these averages. Comparisons of paired variables at the different heart rates were performed with a paired two-tailed t-test. A value of P<0.05 was considered statistically significant.

6.3 Results

Average pressures, flows, heart rate and oxygen consumption are provided in Table 6.1. Data from control and maximal vasodilatory conditions are grouped according to heart rate. After maximal vasodilation with adenosine, flow had increased and perfusion pressure decreased compared to control. On average the flow step was about 24% of total flow. The resulting steady state change in O₂ consumption was about 8%, only during vasodilation and at a heart rate of 130 beats/min the change in oxygen consumption was 13%. As expected, average oxygen consumption increased with increasing heart rate.

A typical time response of the arterio-venous O₂ content difference to a step change in arterial flow during control is illustrated in Figure 6.1. In this figure the average values per heart beat are provided. At t=15 s the arterial flow was changed stepwise. The response of the AVo₂ is characterized by 2 stages: 1. a time delay and 2. a slow change to a new steady state value. Left ventricular pressure remained constant, whereas the perfusion pressure increased due to the increased flow, and later as a result of the increased vascular resistance due to autoregulation.

Figure 6.2 provides the estimated volumes using a time constant of 6 s for change in O₂ consumption. For each goat the control and vasodilation
measurements were grouped according to heart rate. The resulting average volumes per goat were averaged in the same manner to obtain the overall volumes per heart rate for control and vasodilation. The reported mixed volumes were corrected for the influence of changes in tissue oxygen stores. This correction term varied between 0.9 and 2.4 ml/100 g depending on hemoglobin content and working point for the linearization of the Po2 saturation curves. During control no effect of heart rate on either mixed or unmixed volume was found. However, during maximal vasodilation an increment of heart rate resulted in an increase in estimated mixed volume, whereas no effect on unmixed volume was found. A paired student t-test indicated that only a heart rate variations between 60 and 130 beats/min affected mixed volume significantly (P<0.02). Administration of adenosine significantly increased the mixed volume compared to control at heart rates of 90 and 130 beats/minute (P<0.05 and P<0.01, respectively), whereas unmixed volume was increased only at a heart rate of 60 beats per minute (P<0.01). The direction of the flow steps did not affect the volume estimates during control. However, during maximal vasodilation mixed volumes apparently are higher after flow increases compared with flow decreases.
Table 6.1 Hemodynamic data for control and maximal vasodilation grouped according to heart rate, and heart weight.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maximal vasodilation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 60, n=6</td>
<td>HR 90, n=4</td>
</tr>
<tr>
<td>$P_{N\text{-SYS}}$ [mmHg]</td>
<td>64 ± 5</td>
<td>71 ± 11</td>
</tr>
<tr>
<td>$P_{P}$ [mmHg]</td>
<td>101 ± 8</td>
<td>96 ± 11</td>
</tr>
<tr>
<td>$P_{\text{epic.ven.}}$ [mmHg]</td>
<td>7.3 ± 1.6</td>
<td>9.3 ± 1.8</td>
</tr>
<tr>
<td>$Q_{a}$ [ml/s/100 g]</td>
<td>1.6 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>$Q_{v}$ [ml/s/100 g]</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td>$MVO_{2}$ [μl O₂/s/100 g]</td>
<td>71 ± 10</td>
<td>83 ± 8</td>
</tr>
<tr>
<td>$C_{MVO_{2}/C_{O_{2}}}$ ratio [%]</td>
<td>31 ± 4</td>
<td>31 ± 4</td>
</tr>
</tbody>
</table>

- **Heart weight [g]**:
  - total: 135 ± 8
  - left ventricle: 80 ± 6
  - perfused area: 79 ± 5

Means ± SE. n: number of goats, HR: heart rate, $P_{N\text{-SYS}}$: systolic left ventricular pressure, $P_{P}$: perfusion pressure, $P_{\text{epic.ven.}}$: epicardial venous pressure, $Q_{a}$: coronary arterial flow, $Q_{v}$: coronary venous flow, $MVO_{2}$: myocardial oxygen consumption, $C_{MVO_{2}/C_{O_{2}}}$ ratio: relative contribution of $MVO_{2}$ and flow change to the change in $AVO_{2}$ (see Methods and Discussion).

**Figure 6.2** Average estimated volume distribution ± SE per heart rate during control and maximal vasodilation. Left panel: mixed volume, right panel: unmixed volume. Data were obtained assuming an exponential change in oxygen consumption with a time constant of 6 s. *: significantly different from control, □: significantly different from HR 60.
Figure 6.3 Estimated mixed volume per animal plotted versus heart rate. Left panel: control, right panel: maximal vasodilation. Data were obtained assuming an exponential change in oxygen consumption with a time constant of 6 s. Each symbol represents a different animal.

Figure 6.4 Dependency of the average volume estimations on the assumed oxygen consumption time course. Oxygen consumption was assumed to change exponentially with a time constant of 0 (○), 3 (∆), 6 (□) or 9 (◇) s. The effect is most prominent on the estimation of mixed volume, especially during maximal vasodilation (filled symbols) at high heart rate. Bars represent SE. For clarity, bars have not been drawn for each value.

at a heart rate of 130 beats/min (27.3±4.4 versus 20.4±3.3, P<0.02).

In Figure 6.3 the individual average mixed volumes for each goat are plotted against heart rate to show the variation between the animals. Especially during vasodilation (Figure 6.3, right panel) the interindividual variation is large.

The effect of different time constants (0, 3, 6 and 9 s) for the assumed exponential oxygen consumption time course on the volume determination is illustrated in Figure 6.4. An effect of the time constant is most prominent on the estimations of the mixed volume (see also Table 6.2). Larger time constants for
Table 6.2 The effect of the time constant for the exponential oxygen consumption change on the estimated mixed volume [ml/100 g] per heart rate (HR) during control and maximal vasodilation.

<table>
<thead>
<tr>
<th>Time constant</th>
<th>Control</th>
<th>Vasodilation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 60</td>
<td>HR 90</td>
</tr>
<tr>
<td></td>
<td>n=6</td>
<td>n=4</td>
</tr>
<tr>
<td>0</td>
<td>6.3 ± 0.5</td>
<td>5.8 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>8.0 ± 0.5</td>
<td>7.3 ± 1.2</td>
</tr>
<tr>
<td>6</td>
<td>9.5 ± 0.8</td>
<td>8.6 ± 1.6</td>
</tr>
<tr>
<td>9</td>
<td>10.6 ± 1.1</td>
<td>9.3 ± 1.7</td>
</tr>
</tbody>
</table>

Means of n goats ± SE.

the exponential oxygen consumption change, indicating slower changes, result in higher mixed volumes. Especially during vasodilation and a heart rate of 130 beats per minute this effect is large.

From the integral of the difference between arterial flow and scaled venous flow, changes in total coronary volume could be estimated (Section 2.2.6). With this method, volume changes induced by the heart rate changes between the flow steps were calculated. Coronary volume decreased by 0.38±0.06 ml/100 g during control when heart rate was changed from 60 to 130 beats/min, whereas during maximal vasodilation coronary volume decreased 0.77±0.09 ml/100 g. These decreases in coronary blood volume with increasing heart rate are in disagreement with the results on the volumes estimated from the A\textsubscript{VO}_2 transients induced by the flow steps.

\( C_{\text{MV}O_2}/C_{Qa} \) ratios (see Methods, Equation 6.14) were calculated for each intervention and averaged per goat after grouping to heart rate and control versus vasodilation. In Figure 6.5 these data are plotted versus the average mixed volume. High values for mixed volume were only obtained with high \( C_{\text{MV}O_2}/C_{Qa} \) ratios. Although less clear and with a larger spread, this pattern is also present within the individual animals. These results suggest that the volume estimation method fails when the \( C_{\text{MV}O_2}/C_{Qa} \) ratio is larger than 0.4. Hence, for each goat the interventions with \( C_{\text{MV}O_2}/C_{Qa} \) ratios smaller than 0.4 were analyzed separately. The results are given in Figure 6.6. Compared with the results without selection on \( C_{\text{MV}O_2}/C_{Qa} \) ratio (Figure 6.4), the dependance of mixed vessel volume on the assumed MV\textsubscript{O}_2 time course has reduced, especially during maximal vasodilation. Unmixed volume was less affected by the selection on \( C_{\text{MV}O_2}/C_{Qa} \) ratio. However, during vasodilation the mixed vessel volume and the sum of \( V_m \) and \( V_{unm} \) still increases significantly with HR. Hence, a discrepancy between the results based on the A\textsubscript{VO}_2 simulation method and the coronary volume changes measured from the difference between in- and outflow remains.
Figure 6.5 Mixed volume versus the relative contribution of the oxygen consumption change to the AVo₂ change compared to the flow change contribution (C_MVo₂/C_Qa). High values for this ratio indicate less reliable estimations of coronary volume. Each point represents an average for one animal at a certain heart rate during control (open symbols) or vasodilation (filled symbols). Mixed volume was estimated with an exponential oxygen consumption time course with a time constant of 6 s. ●: HR 60, △: HR 90, ○: HR 130, ▽: HR 175.

C_MVo₂/C_Qa < 0.4

Figure 6.6 Dependency of the average volume estimations on the assumed oxygen consumption time course. Only interventions with C_MVo₂/C_Qa ratios < 0.4 are used for this figure. Oxygen consumption was assumed to change exponentially with a time constant of 0 (●), 3 (△), 6 (○) or 9 (▽) s. Compared with the unselected data (Figure 6.4), the effect of the MVo₂ time course is smaller. Bars represent SE. For clarity, bars have not been drawn for each value. Open symbols: control, filled symbols: vasodilation.
6.4 Discussion

6.4.1 Dependency of estimated volume distribution on the change in myocardial oxygen consumption

An oxygen exchange model was used to estimate the distribution of coronary volume in vessels participating in the oxygen exchange and venous transport vessels. The method is based on comparison of measured $\text{AVo}_2$ responses to a flow step with simulations. To simulate $\text{AVo}_2$ responses, the oxygen consumption time course should be known. However, myocardial $\text{O}_2$ consumption can only be measured during steady conditions before and after the flow step, whereas the time course of transients can only be assumed. Hence, while the volume estimation method should give reliable estimates when $\text{O}_2$ consumption is constant, an error will be introduced with changing $\text{O}_2$ consumption when the assumed and actual $\text{MVo}_2$ time courses differ. Obviously this effect becomes more important when the change in $\text{O}_2$ consumption is larger.

The error in the volume estimations caused by $\text{O}_2$ consumption changes was investigated using simulations. The $C_{\text{MVo}_2}/C_{\text{Qa}}$ ratio (see Methods) was used as a measure of the importance for the $\text{AVo}_2$ transient of the $\text{MVo}_2$ change compared to the flow change. With the model, $\text{AVo}_2$ responses were simulated using a step change in $\text{MVo}_2$ from 70 to 76 $\mu\text{l} \text{O}_2/\text{s}/100 \text{ g}$ and $C_{\text{MVo}_2}/C_{\text{Qa}}$ ratios of 10, 30, 50 and 70%. $C_{\text{MVo}_2}/C_{\text{Qa}}$ ratios were varied by varying the magnitude of the flow step while the flow after the flow step was taken constant (1.8 ml/s/100 g). Mixed volume was 10 ml/100 g, and unmixed volume was 0 ml/100 g. The four simulations were treated as measured $\text{AVo}_2$ responses, and the volume distribution was estimated. Results of the simulations are depicted in Figure 6.7. For the four simulations, oxygen consumption was assumed to change stepwise. Therefore, errors in the volume distribution estimation are absent when a step change in oxygen consumption ($\tau_{\text{MVo}_2}=0$ s) is assumed in the analysis. By taking a time constant in the fitting procedure different from the original time constant, errors in the estimated mixed volumes occur. These errors increase more than linear with the $C_{\text{MVo}_2}/C_{\text{Qa}}$ ratio. The magnitude of the error is dependent on the time course assumed in the model fit. The simulations also demonstrate the weak interaction between the errors in mixed volume and unmixed volume in our procedure of volume estimations. The similarity between the dependency of the experimentally found mixed volumes on the $C_{\text{MVo}_2}/C_{\text{Qa}}$ ratio (Figure 6.5) and the simulation results (Figure 6.7, left panel, $\tau_{\text{MVo}_2}=6$ s) makes it plausible that the $C_{\text{MVo}_2}/C_{\text{Qa}}$ ratio is an important determinant of the error in the mixed volume estimation.

The average $C_{\text{MVo}_2}/C_{\text{Qa}}$ ratio during vasodilation at HR 130 was largest (see Table 6.1). This explains the large dependency on the assumed $\text{MVo}_2$ time
course of the experimental data obtained under these circumstances (Figure 6.4 and Table 6.2). However, it should be noted that errors in the estimation of the volume distribution only occur when the assumed oxygen consumption time course is incorrect. The assumed MVo₂ time course with a time constant of 6 s is not unrealistic considering the results from studies on the rate of myocardial oxygen consumption changes after a change in heart rate (Vergroesen and Spaan, 1988; Van Beek and Westerhof, 1991). The similarity between the experimental and simulation results suggest that the MVo₂ time course may be faster. Average volume estimations obtained after a selection of the data with small C_{MVo₂}/C_{Qa} ratios were less dependent on the assumed MVo₂ time course (compare Figures 6.4 and 6.6). However, for all assumed MVo₂ time courses, during vasodilation the sum of mixed vessel volume and unmixed volume still increased about 5 ml/100 g when heart rate was increased from 60 to 130 beats/min. Hence, the disagreement with the measurements on coronary volume change after a heart rate change remains present, even when assuming an unrealistically fast oxygen consumption change.

To explain these conflicting results, the effects of flow level, direction of the flow change and discretisation times on the volume estimations have been investigated. However, no significant effects of these variables were found.

A possibility for the explanation of the increased volume estimations may be that the time courses of the oxygen consumption changes are different at the different heart rates. However, one may only speculate on such a difference. An
alternative explanation is that the assumption of a well-mixed compartment is too much of an oversimplification when flow and oxygen consumption change concomitantly.

6.4.2 Conclusions

The techniques available to us allowed estimation of a coronary volume distribution only on the basis of a model of myocardial oxygen exchange. Quite conventionally we have assumed a well-mixed oxygen exchange compartment. We have examined many possible factors deviating our model assumptions from reality (Chapter 3). However, we have not been able to find a satisfactory explanation why, only during vasodilation, at high heart rate blood volume in the O₂ exchange compartment would be higher than at low heart rate. This finding is in contradiction to the results from the integral of the difference in arterial and venous flow, which indicate that coronary blood volume decreases with a heart rate increase. This discrepancy indicates the limits of our present concepts on oxygen exchange and distribution in the heart.
7

Conclusions

The thesis clearly demonstrates the interaction between heart contraction, oxygen consumption and perfusion: heart contraction determines oxygen consumption and thereby myocardial perfusion, whereas perfusion in itself affects myocardial oxygen consumption.

These interactions complicate the study of myocardial perfusion and its control. In steady state the different mechanisms can not be untangled since too few input and output relations can be found. Dynamic analysis is complicated, since the different phenomena change with time constants of the same magnitude. We were lucky that by administration of glibenclamide the rate of coronary tone adjustments could be decreased, which allowed us to study the perfusion induced oxygen consumption independent of the oxygen consumption required for cardiac performance (Chapter 5). However, the interactions restricted us in estimating especially coronary blood volume distribution by the use of hemoglobin-bound oxygen as an endogenous tracer. This method is based on measurements of the transient in oxygen extraction induced by a step change in perfusion (Chapters 2 and 6). The method of analyzing was designed under the assumption that oxygen consumption would be constant. However, as explained in Chapter 4, oxygen consumption changes when perfusion changes with an unknown time course. Obviously, as long as oxygen consumption changes are relatively small, the error induced can still be corrected for, but not when these are large. It appeared that, especially at high heart rates and maximal vasodilation, the change in perfusion induced oxygen consumption is too large to be compensated for without detailed knowledge of the time course of the oxygen consumption (Chapter 6).

This era of coronary circulation research is one of conceptual changes. It has become clear that dynamic changes of flow with time constants smaller than the duration of a heart beat cannot be considered without capacitive components. The effect of contraction on coronary perfusion seems to be prescribed by the filling volume of the intramyocardial vessels: the larger the filling the more intravascular blood pressure will be generated by contraction (elastance effect). It is also appreciated that resistance of the vascular bed is lower at increased volume. Hence, intramural blood volume has been receiving an increasing amount of attention in recent research.

In fact, the genesis of this thesis had the estimation of volume distribution in mind. The techniques available to us allowed estimation of this distribution only on the basis of a model of oxygen distribution in myocardial tissue.
However, in this area concepts are developing as well. Quite traditionally we have assumed a well-mixed oxygen exchange compartment. Although we have analyzed many possible factors deviating our model assumptions from reality (Chapter 3), we have not been able to come up with a satisfactory explanation why at high heart rate blood volume in the exchange compartment would be higher than at low heart rate, and only during vasodilation. This is even more striking since, from the integral of the difference in arterial and venous flow, we would conclude a decrease in blood volume with a heart rate increase. This discrepancy indicates the limits of our present concepts on oxygen exchange and distribution in the heart.
Summary

The heart is in permanent need of oxygen and nutrients in order to maintain its pumping action. These substances are supplied by blood flowing through the coronary circulation. Contraction of the heart muscle impedes the flow through these blood vessels. Hence, at an increased heart rate flow impediment is larger, while the metabolic demands of the muscle increase. In healthy hearts the increased flow impediment is compensated by a decrease in coronary resistance caused by the adjustment of the diameters of small arteries. However, in patients with coronary artery disease coronary resistance in large coronary arteries is increased, which must be compensated for by vasodilation of the small arteries. For these patients, the possibilities left for adjustment of coronary vessel diameters are small. Hence, especially at high heart rates coronary flow cannot be adapted sufficely to fulfil the demand, and a myocardial infarction may occur.

In this thesis, the dependance of myocardial oxygen consumption on coronary flow and pressure is analyzed. Furthermore, a method has been developed to determine the volume of the vessels participating in oxygen exchange. Knowledge of oxygen consumption determinants and coronary volume are important to clarify the exact mechanisms of the mechanical flow impediment by the heart contraction and of the adjustment of coronary resistance to the metabolic demands.

In Chapter 2 a method is presented to estimate the volume of the vessels participating in oxygen exchange and the volume of the venous vessels with a transport function. In anesthetized goats, the left main coronary artery was cannulated and perfused from an external circuit. The great cardiac vein was also cannulated and drained into a reservoir under constant pressure. The coronary circulation was perfused with a constant flow. The response of the difference in oxygen content between the arterial and venous blood to an abrupt flow change was measured and compared with simulations from a two compartment model representing the coronary circulation. The volumes of the two compartments are the parameters to be estimated. The model is based on the mass balance of oxygen: the change of the amount of oxygen in a compartment is equal to the difference between the amount of oxygen entering the compartment by blood flow and the amount of oxygen leaving the compartment by blood flow and oxygen consumption. Hence, arterial flow and myocardial oxygen consumption content are input signals for the model. The method was based on the assumption of a relatively constant oxygen consumption. However, the flow step induced a significant change in oxygen consumption. Although oxygen consumption can be measured under steady conditions, the time course of the oxygen consumption
transient cannot be measured. It was assumed to be exponential with a time constant based on values obtained from the literature. By this method, volumes were estimated at a heart rate of 90 beats/min during control and after maximal vasodilation with adenosine. The results were 9.9 and 3.8 ml/100 g for the O₂ exchange vessels and the distal venous volume, respectively, during control and 13.1 and 5.0 ml/100 g during maximal vasodilation. Hence, the increase in total blood volume induced by vasodilation should be looked for in the capillary bed and not in the coronary veins. Furthermore, the time constant involved in reaching equilibrium of tissue oxygen tension at relatively constant metabolism follows from the ratio between the oxygen stored in blood and tissue and the oxygen supply, which is represented by the ratio of the first compartment volume and coronary arterial flow. From our experiments, the values of 8.3±0.9 and 6.5±0.5 s (means±SE) for control and vasodilation, respectively, were calculated for these time constants.

The oxygen exchange model is a simplified presentation of the coronary circulation, consisting of a well-mixed compartment from which oxygen is consumed and an unmixed compartment. It does not include phenomena such as flow heterogeneity, different mixing sites or Krogh-like oxygen exchange. However, in Chapter 3 models which include these factors were evaluated. Volumes estimated using the more realistic versions of these models differed only slightly from the results of the original model. Therefore, these results support the idea that the original model can be used to estimate coronary volume distribution.

In Chapter 4 the effect of coronary perfusion on the myocardial oxygen consumption was analyzed. This effect is named after its discoverer: Gregg's phenomenon. In the anesthetized goat preparation as was described above, flow steps were applied at different heart rates during control and maximal vasodilation. Myocardial oxygen consumption was calculated in the steady conditions before and after the flow step by multiplication of flow and arterio-venous oxygen content difference (Fick's law). It was found that Gregg's phenomenon depends on heart rate: at higher heart rate the dependency of myocardial oxygen consumption on flow increases.

In Chapter 5, the model to estimate the coronary volume distribution, as described above, was used to calculate the time course of changes in myocardial oxygen consumption. In the anesthetized goat preparation, the coronary circulation was perfused under constant pressure during control and after administration of the K⁺-blocker glibenclamide, which decreases the rate of regulation by about a factor 4. The calculated transients of oxygen consumption depend on the assumed values for the compartment volumes and are therefore only global estimates of the actual time courses. However, during control the response was clearly very different from the time course after glibenclamide
administration. The time course of myocardial oxygen consumption appeared to depend to a large extent on the rate of regulation. Both this finding and the stronger flow dependency of oxygen consumption at high heart rates than at low heart rates can be explained by a dependence of myocardial oxygen consumption on the capillary pressure. This dependence has already often been stated as a cause of Gregg's phenomenon.

In Chapter 6, the dependency of the coronary volume distribution on heart rate was estimated using the method described in Chapter 2. Again, measurements were obtained from the anesthetized goat under constant flow perfusion at different heart rates. Independent of the assumed oxygen consumption time course, during control an effect of heart rate on the volume of the vessels participating in O₂ exchange and the volume of the venous transport vessels within the sensitivity of the described method was not found. Hence, we may state that, although heart contraction affects intramyocardial volume, during control the differences in volume distribution between heart rates of 60 and 130 beats/min are only minor. This was confirmed by results on calculations of the coronary volume change immediately after a heart rate step from the integrated difference between arterial and venous flow. During control, the volume decreased 0.4 ml/100 g with a heart rate increase, whereas during maximal vasodilation a decrease of 0.8 ml/100 g was found. However, using the simulation method, during maximal vasodilation an increase in total coronary volume was found when heart rate increased. This increase was partly explained by the large change in myocardial oxygen consumption at high heart rates during maximal vasodilation, since a deviation between assumed and actual oxygen consumption time course will cause a larger error in the volume estimations when the oxygen consumption change is large. However, after selection of interventions with smaller oxygen consumption changes, the volume increase with heart rate became smaller but was still present. We have not been able to find a satisfactory explanation for this volume increase with heart rate, which has only been found during vasodilation. Erroneous assumptions on the oxygen consumption time course may be a cause of these findings, however, only speculations can be made on this time course. Another cause may be that the assumption of a well-mixed compartment for oxygen exchange is too much of a simplification when coronary flow and myocardial oxygen consumption change simultaneously. The traditional concept of a well-mixed oxygen exchange compartment should therefore be reconsidered.
Samenvatting

Het hart heeft een continue behoefte aan zuurstof en voedingsstoffen om het bloed door het lichaam te kunnen blijven pompen. Deze stoffen worden geleverd via bloed dat stroomt door een stelsel van bloedvaten in de hartspier zelf: de coronaire circulatie. Het samentrekken van het hart belemmert de bloedstroom door deze vaten. Bij een verhoogde hartfrequentie is de stroombelemmering groter, terwijl de behoefte aan voedingsstoffen van het hart is toegenomen. In gezonde harten wordt de toegenomen stroombelemmering gecompenseerd door een afname van de coronaire weerstand door aanpassing van de diameters van de kleine toevoervaten (arteriën). Bij hartpatiënten is de weerstand in de grote arteriën (kransslagaderen) vaak toegenomen. Dit moet gecompenseerd worden door een verwijding van de kleine arteriën. Deze patiënten hebben daarom een verminderde mogelijkheid om de diameters van de coronaire arteriën verder aan te passen. Daardoor kan met name bij een hoge hartfrequentie de bloedstroom door de coronaire circulatie niet voldoende worden aangepast aan de behoefte. Dit kan een hartinfarct tot gevolg hebben.

In dit proefschrift is de afhankelijkheid van de zuurstofconsumptie van het hart voor de bloedstroom en de bloeddruk onderzocht. Verder is een methode ontwikkeld om het volume van de bloedvaten die belangrijk zijn voor de zuurstofuitwisseling te bepalen. Kennis over de determinanten van de zuurstofconsumptie van het hart en het coronaire volume is belangrijk om de precieze mechanismen van de mechanische bloedstroombelemmering door de hartcontractie en de aanpassing van de coronaire weerstand aan de vraag om bloed te achterhalen.

In hoofdstuk 2 is een methode gepresenteerd om het volume van de bloedvaten die belangrijk zijn voor de zuurstofuitwisseling en het volume van de veneuze vaten met een transportfunctie te bepalen. In genarcotiseerde geiten is de linker hoofdkransslagader gecanneleerd en doorstroomd via een pompstelsel. De grote hartvene is ook gecanneleerd en het bloed uit deze vene stroomde naar een reservoir met een constante druk. De bloedstroom naar de coronaire circulatie werd constant gehouden. De responsie van het verschil in arteriële en veneuze zuurstofconcentratie na een snelle verandering van de bloedstroom is gemeten en vergeleken met simulaties uit een twee-compartmenten model dat de coronaire circulatie voorspelt. De volumes van de twee compartimenten zijn de modelparameters die geschat moeten worden. Het model is gebaseerd op de massa-balans van zuurstof: de verandering van de hoeveelheid zuurstof in een compartiment is gelijk aan het verschil tussen de zuurstof toevoer via de bloedstroom en de zuurstofafvoer via de bloedstroom en de zuurstofconsumptie. De bloedstroom en de zuurstofconsumptie van het hart zijn daarom ingangs-
signalen van het model. De methode is gebaseerd op een relatief constante zuurstofconsumptie. Echter, door de bloedstroomverandering veranderde de zuurstofconsumptie aanzienlijk. De zuurstofconsumptie kan in evenwichtssituaties worden gemeten, maar de verandering van de zuurstofconsumptie in de tijd kan niet gemeten worden. Daarom is een exponentiële verandering met een tijdconstante gebaseerd op waarden uit de literatuur aangenomen. Met deze methode werden volumes geschat bij een hartfrequentie van 90 slagen per minuut tijdens normale omstandigheden en na vaatverwijding met adenosine. Tijdens normale omstandigheden waren de resultaten 9.9 ml/100 gram weefsel voor de vaten met zuurstofuitwisseling en 3.8 ml/100 g voor het venuzeuze volume. Tijdens vaatverwijding waren deze waarden respectievelijk 13.1 en 5.0 ml/100 g. De toename in het coronaire vaatvolume door vaatverwijding moet dus gezocht worden in het capillaire vaatbed en niet in de venen. Verder kan bij een relatief constant metabolisme de tijdconstante voor het bereiken van een evenwicht in zuurstofspanning in het weefsel bepaald worden uit de ratio van de hoeveelheid zuurstof in bloed en weefsel ten opzichte van de zuurstoftoevoer, wat overeenkomt met de verhouding van het volume van het eerste compartiment en de bloedstroom. Uit onze metingen werden tijdconstanten gevonden van 8.3 s tijdens normale omstandigheden en 6.5 s tijdens vaatverwijding.

Het model voor zuurstofuitwisseling is een vereenvoudigde voorstelling van de coronare circulatie, dat bestaat uit een goed-gemengd compartiment van waaruit de zuurstofconsumptie plaats vindt, en een ongemengd compartiment. Verschijnseelen als een heterogene bloedstroomverdeling, meerdere plaatsen met zuurstofmenging of een Krogh-achtige zuurstofuitwisseling zijn niet in het model opgenomen. In hoofdstuk 3 zijn modellen waarin deze factoren werden aangebracht onderzocht. Volumes die geschat werden met de realistische versies van deze modellen verschillen maar weinig van de resultaten met het oorspronkelijke model. Daarom ondersteunen deze resultaten het idee dat het oorspronkelijke model gebruikt kan worden voor het bepalen van de coronare volumeverdeling.

In hoofdstuk 4 is het effect van de coronare doorstroming op de zuurstofconsumptie van het hart geanalyseerd. Dit effect is genoemd naar zijn ontdekker: het Gregg-effect. In de genarcotiseerde geit werden stapvormige bloedstroomveranderingen gegeven bij verschillende hartfrequenties tijdens normale omstandigheden en tijdens maximale vaatverwijding. De zuurstofconsumptie van het hart werd berekend door de bloedstroom te vermenigvuldigen met het arterio-venuze zuurstofverschil (de wet van Fick). Uit de resultaten bleek dat het Gregg-effect afhankelijk is van de hartfrequentie: bij een hogere hartfrequentie is de afhankelijkheid van de zuurstofconsumptie van het hart aan de bloedstroom groter.

In hoofdstuk 5 is het genoemde model van de coronare circulatie gebruikt
om het verloop van de zuurstofconsumptie van het hart in de tijd te berekenen. In de genarcotiseerde geit werd de coronaire circulatie doorstroomd onder een constante druk tijdens normale omstandigheden en na het toedienen van glibenclamide, een stof die de $K^+$-ATP-kanalen blokkeert en daardoor de snelheid van regeling met ongeveer een factor 4 vertraagt. Het berekende tijdsverloop van de zuurstofconsumptie hangt af van de waarden die zijn aangenomen voor de volumes van de compartimenten in het model, en is daarom alleen een globale afschatting van het werkelijke tijdsverloop. Het tijdsverloop tijdens normale omstandigheden was echter duidelijk verschillend van het tijdsverloop na glibenclamide-toediening. Het tijdsverloop van de zuurstofconsumptie van het hart bleek voor een groot deel af te hangen van de snelheid van de regeling. Dit resultaat en de sterkere bloedstroomafhankelijkheid van de zuurstofconsumptie bij hogere hartfrequenties kunnen beide worden verklaard door een afhankelijkheid van de zuurstofconsumptie van het hart aan de capillaire druk. Deze invloed van de capillaire druk is al vaak genoemd als mogelijke oorzaak van het Gregg-effect.

In hoofdstuk 6 is geprobeerd te bepalen hoe de coronaire volumeverdeling door de hartfrequentie beïnvloed wordt. De methode uit hoofdstuk 2 werd hiervoor gebruikt. Opnieuw werden metingen verricht bij de genarcotiseerde geit met een constante coronaire bloedstroom tijdens verschillende hartfrequenties. Onafhankelijk van de veronderstelling over het verloop van een zuurstofconsumptieverandering in de tijd, werd binnen de nauwkeurigheid van de methode tijdens normale omstandigheden geen effect gevonden van de hartfrequentie op het volume van de vaten die zorgen voor de zuurstofuitwisseling en het volume van de veneuze vaten. We kunnen dus concluderen dat tijdens normale omstandigheden het effect van een verandering in de hartfrequentie van 60 naar 130 slagen per minuut het coronaire volume hooguit weinig beïnvloedt. Dit werd bevestigd door resultaten van bepalingen van coronaire volumeveranderingen na een hartfrequentieverandering uit het geïntegreerde verschil van de arteriële en de veneuze bloedstroom. Na een hartfrequentieverhoging, nam tijdens normale omstandigheden het totale volume van het coronaire vaatbed 0.4 ml/100 g af, terwijl tijdens maximale vaatverwijding een afname van 0.8 ml/100 g werd gevonden. Met de simulatiemethode werd echter tijdens maximale vaatverwijding een toename in de som van de twee compartimentvolumes gevonden. Deze toename kon gedeeltelijk verklaard worden door de grote zuurstofconsumptieverandering tijdens hoge hartfrequenties en maximale vaatverwijding. Een verschil tussen het werkelijke en het aangenomen verloop van de zuurstofconsumptieverandering zal bij een grote zuurstofconsumptieverandering een sterkere fout in de volumeschattingen geven. Echter, na selectie van metingen met een relatief kleine zuurstofconsumptieverandering, werd de volumetoename bij een hartfrequentie-verhoging
wel kleiner, maar hij was nog steeds aanwezig. Er is geen bevredigende verklaring gevonden voor het resultaat dat alleen bij maximale vaatverwijding bij een hoge hartfrequentie een groter bloedvolume in het compartiment met zuurstofuitwisseling wordt geschat dan bij lage hartfrequentie. Mogelijk ligt de oorzaak in een verkeerde veronderstelling van het verloop van de veranderende zuurstofconsumptie, echter, over dit verloop kan alleen gespeculeerd worden. Een andere oorzaak kan zijn dat het veronderstellen van een goedgemengd zuurstofcompartiment een te eenvoudige voorstelling is wanneer de bloedstroom en de zuurstofconsumptie beide tegelijkertijd veranderen. De huidige inzichten in de zuurstofverdeling en -consumptie in het hart schieten onder deze omstandigheden tekort.
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