Metabolic flow regulation in human coronary artery disease
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Chapter 5

Metabolic coronary flow regulation and exogenous nitric oxide in human coronary artery disease

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Abstract

Objective. To evaluate the effect of intravenous administration of the nitric oxide donor substance nitroglycerin (NTG) on metabolic coronary flow regulation in patients with coronary artery disease (CAD).

Methods. In twelve patients with stable CAD, we measured coronary sinus blood flow, myocardial oxygen supply and consumption (MVO₂) at sinus rhythm and during atrial pacing (30 bpm above sinus rate), both at control, and during infusion of NTG, 1 µg/kg/min, and NTG, 2 µg/kg/min. To study metabolic coronary vasodilation, changes in myocardial oxygen supply were related to pacing induced changes in MVO₂, using standard regression analysis. The myocardial oxygen supply-consumption ratio, i.e. the slope of the regression line at control, characterizing physiological metabolic coronary flow regulation, was compared with the ratios obtained during infusion of NTG.

Results. Compared to control measurements, NTG 1 µg/kg/min and NTG 2 µg/kg/min attenuated pacing induced increases in MVO₂ by 29% and 60% respectively, while coronary blood flow during pacing remained unchanged. At control, normal metabolic coronary flow regulation resulted in a myocardial oxygen supply-demand ratio of 1.39 (95% CI: 1.29 - 1.49). This ratio did not change during NTG 1 µg/kg/min: 1.44 (95% CI: 1.33 - 1.56). However, during NTG 2 µg/kg/min, this ratio significantly increased to 1.84 (95% CI: 1.63 - 2.05; p<0.01).

Conclusions. Intravenous administration of high dose NTG, a donor of exogenous NO, blunts pacing induced increases in MVO₂ and may increase metabolic coronary vasodilation in patients with CAD.
1. Introduction

Coronary blood flow is largely dependent on myocardial oxygen consumption (MVO₂), a process known as metabolic coronary flow regulation. Endothelium derived nitric oxide (NO) supposedly plays an important role in this regulation process, since the coronary vasodilation in response to pacing induced increases in MVO₂ was reported to be attenuated after blockade of endogenous NO synthesis in both dogs [1,2] and humans [3-5], although some studies failed to demonstrate such a role for NO [6,7]. In patients with coronary artery disease (CAD), epicardial and microvascular vasomotor responses are attenuated [3,7-11], which may be explained by the reduced endothelial NO release in those patients [12].

Since organic nitrates produce their biological effect by releasing NO and thus in many respects mimic the effect of endogenous NO [13], they have been used as NO donors [14]. Whether supplementation of exogenous NO by organic nitrates improves the attenuated metabolic coronary vasodilation in patients with CAD, is not well defined. To date, the effect of nitrates on the adaptation of coronary blood flow in response to metabolic stimuli has only been studied during myocardial ischemia, induced by rapid pacing or exercise [15-17]. Interpretation of these studies is difficult, since during myocardial ischemia, other than metabolic coronary flow regulating mechanisms may prevail [18].

To investigate the role of exogenous NO in mediating coronary vascular responses to a metabolic stimulus in patients with CAD, we examined the coronary vascular responses to small pacing induced changes in HR before and after the administration of the NO donor substance nitroglycerin (NTG).

2. Methods

2.1 Patients
The study group consisted of 12 patients (all men) with stable CAD, without clinical or echocardiographic evidence of congenital, valvular or hypertrophic heart disease. All patients were scheduled for elective coronary artery surgery and were enrolled in the study when left ventricular and coronary angiography revealed a left ventricular end diastolic pressure lower than 18 mmHg, a left ventricular ejection fraction higher than 45% and the absence of a left main stem stenosis. Patients with atrioventricular conduction defects or unstable angina were excluded from the study. Calcium channel blockers and long acting nitrates were discontinued the evening before surgery, whereas only β-adrenoceptor blockers were given until the morning of surgery. Premedication consisted of lorazepam 4-5 mg orally, which was given two hours before start of the study. All patients gave written informed consent to participate in this study which was approved by the local ethical committee and complied with the principles outlined in the Declaration of Helsinki.

2.2 Instrumentation
On arrival in the operating room, ECG leads were connected and lead II, III and V5 were continuously monitored (HP Merlin System, Hewlett-Packard, Böblingen,
Germany). A wide bore peripheral venous catheter and a 20-gauge radial artery catheter were inserted under local analgesia. A thermodilution pulmonary artery catheter (Baxter Health Care Corporation, Irvine, California, USA) and a coronary sinus thermodilution catheter (Wilton-Webster Laboratories, Altadena, California, USA) were introduced via the left subclavian vein. The coronary sinus catheter was advanced into the coronary sinus using image intensification fluoroscopy and injection of contrast medium, so that the external thermistor lay 1.5-2 cm from the ostium and that there was no major sidebranching vein in the vicinity. The coronary sinus catheter was connected to a Wilton Webster Wheatstone bridge. Coronary sinus thermodilution signals were recorded with a multi-channel amplifier/recorder system. Catheter calibration factors provided by the manufacturer were used. The absence of right atrial admixture in coronary sinus blood was checked by injection of cold saline in the right atrium, while coronary sinus temperature curves were recorded simultaneously [19]. Under fluoroscopy, atrial pacing (via coronary sinus catheter) was used during 10-30 seconds to ascertain the stability of the position of the tip of the coronary sinus catheter in relation to the surrounding anatomical structures and fluoroscopic landmarks. If the stability of the catheter could not be guaranteed, the experiment was discontinued. Two patients eligible for the present study, were therefore not included. For the measurement of coronary sinus blood flow (CSBF) normal saline at room temperature was used as indicator and infused into the coronary sinus at a rate of 45 ml/min via a Mark IV infusion pump (Meditronics Technology for people, Pittsburgh, Pennsylvania, USA) [20].

2.3 Measurements

After adequate instrumentation and a resting period of 20 minutes, three series of measurements were performed in each patient. Each series included both an increase in HR (HR step up, induced by pacing) and a decrease in HR (HR step down, induced by the discontinuation of pacing). The first series of measurements was performed before infusion of NTG (control), whereas the second and third series of measurements were performed during infusion of NTG 1 μg/kg/min and NTG 2 μg/kg/min, respectively.

Each series of measurements started at sinus rhythm with the measurement of capillary wedge pressure (PCWP) and cardiac output (CO), and the simultaneous sampling of blood from the radial artery and coronary sinus. Subsequently, continuous recording (digitized on-line at a sampling rate of 80 Hz) of CSBF, arterial blood pressure (ABP), right atrial pressure (RAP), pulmonary artery pressure (PAP) and electrocardiographic lead II was started. Following ten seconds of steady state recording, yielding values at sinus rhythm, HR was increased by 30 beats per minute (bpm) above sinus rate by pacing via the coronary sinus catheter (HR step up). Ten seconds after a new steady state had been reached (usually well within 50 seconds following the HR step), yielding values during pacing, recording was stopped. The total duration of the recordings was about 70 seconds. Subsequently, CO and PCWP were measured during pacing and blood sampling was repeated. Then, during recording of CSBF, ABP, PAP, RAP and ECG, pacing was stopped (HR step down) and recording continued until a new steady state had been recorded, again yielding
values at sinus rhythm. A final series of PCWP and CO measurements and blood sampling completed this series of measurements.

Following the completion of this first series of control measurements, the effect of the NO donor substance NTG on pacing induced changes in coronary blood flow was studied. Patients received an intravenous infusion of NTG at a rate of 1.0 \( \mu g/kg/min \). After an equilibration period of 15 minutes, the measurements series was repeated, with the same pacing rate as was used during control.

In the last ten patients, the infusion rate of NTG was increased to 2.0 \( \mu g/kg/min \), followed by repetition of the measurement series. This high infusion rate of 2 \( \mu g/kg/min \) was chosen to ascertain an effect in patients possibly desensitized to NTG by long acting oral nitrates [13].

2.4 Laboratory analysis and calculations

At each measurement point, thermodilution CO was obtained in triplicate, using normal saline at room temperature as injectate. These three CO values were subsequently averaged and are reported as cardiac index.

Arterial and coronary sinus blood samples were analyzed to determine plasma hemoglobin concentration, oxygen partial pressure (pO\(_2\)) (ABL III, Radiometer, Copenhagen, Denmark), hemoglobin oxygen saturation (SO\(_2\)) (OSM-I hemoxymeter, Radiometer, Copenhagen, Denmark) and lactate concentrations [21].

Myocardial oxygen content was calculated as 1.39 \( \cdot \) Hb \( \cdot \) SO\(_2\) + 2.241 \( \cdot \) 0.00136 \( \cdot \) pO\(_2\) MVO\(_2\) was calculated as CSBF \( \cdot \) arterio-coronary sinus oxygen content difference. Myocardial oxygen supply was calculated as CSBF \( \cdot \) arterial oxygen content. Myocardial lactate uptake (MLU) was calculated as CSBF \( \cdot \) (arterio-coronary sinus lactate concentration). The myocardial lactate extraction percentage was calculated as (arterio-coronary sinus lactate concentration \( \div \) arterial lactate concentration) \( \times \) 100%.

2.5 Data analysis

Using a signal analysis program (Matlab v 3.5j, The MathWorks Inc., Natick, Massachusetts, USA), ABP, PAP, RAP and CSBF were averaged over periods of eight seconds of steady state, before and after the HR step up and down. Therefore two values were obtained during pacing, one after the HR step up, and one before the HR step down. Since these values during pacing were not significantly different, reflecting both the same steady state situation, they were averaged.

The steady state aspects of metabolic flow regulation were analyzed by means of the oxygen supply-demand diagram, showing myocardial oxygen supply as a linear function of MVO\(_2\) (demand), for a given perfusion pressure [22]. As reported previously, we modified this diagram by plotting the changes in oxygen supply against the changes in MVO\(_2\) that were induced by cardiac pacing, to correct for the influence of the variation in coronary perfusion pressure and heart weight [23]. The changes in O\(_2\) supply are related to the changes in MVO\(_2\) by standard regression analysis without intercept, since in response to pacing, a change in myocardial oxygen supply cannot occur without a change in MVO\(_2\), yielding the equation: \( \Delta O_2 \)
supply = ratio · ΔMVO₂. The slope of the obtained line is the reference supply-demand ratio, defining normal metabolic coronary flow regulation [23,24].

In the present study, the reference supply-demand ratio was used to evaluate the effect of NTG on coronary metabolic regulation. This ratio (defining metabolic regulation at control) was therefore compared with supply-demand ratios, obtained from pacing induced changes in supply and consumption, during infusion of NTG 1 μg/kg/min and NTG 2 μg/kg/min (defining metabolic regulation during infusion of NTG). Upward deflection of the regression line, i.e. an increase in the supply-demand ratio, then implies increased metabolic vasodilation, whereas downward deflection means decreased metabolic vasodilation.

The dynamic aspects of metabolic flow regulation were quantified as described earlier [25]. In short, the rate of change of an index of coronary vascular resistance (CRI) in response to a HR step was quantified by a t₅₀-value, which was defined as the time in seconds after the HR step at which the change in CRI had reached 50% of its total change. This value was calculated by fitting a high order polynomial to a part of the signal, over a period of at least 10 seconds, around the 50% value of the CRI-change [25]. In the present study, CRI was calculated as the quotient of beat averaged ABP-RAP and CSBF [25]. Thus, t₅₀ values of HR steps up and HR steps down were obtained at control, and during NTG 1 μg/kg/min and NTG 2 μg/kg/min.

2.6 Statistical analysis
Data obtained at control, during NTG 1 μg/kg/min and NTG 2 μg/kg/min were compared using two-way analysis of variance for repeated measurements. Paired standard t-tests were used to compare values at sinus rhythm to values obtained during pacing. A value of p < 0.05 was considered significant. Results are reported as mean (SD) or as percentage change (SD) where applicable.

Changes in myocardial oxygen supply were related to changes in MVO₂ using standard regression analysis. To compare the slopes of the regression lines obtained at control, during NTG 1 μg/kg/min and NTG 2 μg/kg/min, we used analysis of variance for differences between regression slopes [26]. The slopes of the obtained regression lines, i.e. the myocardial oxygen supply-consumption ratios, are reported with their 95% confidence intervals (95% CI).

3. Results
3.1 Characteristics of the patients
Patients characteristics and pre-operative chronic medication are shown in table 5.1. The patients were all male and comparable with respect to age, weight, and height.

Six patients were suffering from three-vessel CAD, five from two-vessel CAD, whereas only one patient had single-vessel CAD. All were using triple therapy for angina, consisting of long acting nitrates, β-adrenergic blocking agents and calcium channel blockers, except patient 3 who did not use a calcium channel blocker.
### Table 5.1:
Patient characteristics and angiographic findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex (m/f)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Extent of CAD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RCA</td>
</tr>
<tr>
<td>1</td>
<td>m</td>
<td>64</td>
<td>172</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>69</td>
<td>168</td>
<td>71</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>62</td>
<td>168</td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>50</td>
<td>174</td>
<td>84</td>
<td>N</td>
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<td>168</td>
<td>72</td>
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<td>168</td>
<td>92</td>
<td>90</td>
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<td>68</td>
<td>170</td>
<td>82</td>
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<td>m</td>
<td>62</td>
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<td>71</td>
<td>176</td>
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<tr>
<td>12</td>
<td>m</td>
<td>52</td>
<td>170</td>
<td>79</td>
<td>N</td>
</tr>
</tbody>
</table>

*CAD indicates coronary artery disease, reported as percentage narrowing of coronary artery lumen diameter (N denotes normal). RCA, right coronary artery; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery.

### 3.2 Normal metabolic coronary flow regulation: the reference supply-consumption ratio

Pacing the heart at an average 29 bpm above sinus rate at control, resulted in a significant increase in MV0\(_2\) from 13.7 (3.4) ml O\(_2\)/min during sinus rhythm to 19.9 (5.3) ml O\(_2\)/min during pacing (figure 5.1). This increase was completely matched by an increase in CSBF from 104 (30) to 149 (45) ml/min since myocardial oxygen extraction percentage remained unchanged (figure 5.1). Following cessation of pacing, values of MV0\(_2\) and CSBF always returned to pre-pacing values.

Individual changes in myocardial oxygen supply, related to pacing induced changes in MV0\(_2\), are shown in the supply-demand diagram in figure 5.2 (upper panel). Metabolic coronary flow regulation is illustrated by the linear relationship between changes in oxygen supply and consumption. The slope of the calculated regression line (the supply-demand ratio), which is used as reference ratio quantifying metabolic regulation, was 1.39 (95% CI: 1.29-1.49).

### 3.3 NTG during sinus rhythm

The effect of NTG on baseline values of hemodynamic variables at sinus rhythm are shown in table 5.2. In response to NTG 2 µg/kg/min, there was a small reflex increase in sinus rate, associated with the concomitant decrease in mean arterial pressure. In addition, NTG decreased cardiac preload, reflected by a substantial
Table 5.2:
Systemic hemodynamics at sinus rhythm before pacing

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NTG 1 μg/kg/min</th>
<th>NTG 2 μg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=12)</td>
<td>(n=12)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>HR (1/minute)</td>
<td>65 (7)</td>
<td>67 (8)</td>
<td>69 (8)*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>145 (21)</td>
<td>139 (20)*</td>
<td>138 (21)*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>101 (11)</td>
<td>95 (11)*</td>
<td>90 (12)*</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>22 (5)</td>
<td>18 (4)*</td>
<td>17 (3)*</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>7 (3)</td>
<td>5 (3)*</td>
<td>5 (3)*</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>10 (5)</td>
<td>7 (3)*</td>
<td>6 (2)*</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>2.89 (0.38)</td>
<td>2.93 (0.55)</td>
<td>3.00 (0.48)</td>
</tr>
<tr>
<td>SVR (dynes.s/cm²)</td>
<td>1315 (230)</td>
<td>1288 (303)</td>
<td>1193 (267)</td>
</tr>
<tr>
<td>RPP (mmHg/min)</td>
<td>9458 (1696)</td>
<td>9321 (1682)</td>
<td>9556 (1729)</td>
</tr>
</tbody>
</table>

Values are mean (SD). Values at sinus rhythm of heart rate (HR), mean systolic arterial blood pressure (SBP), mean arterial blood pressure (MAP), pulmonary artery pressure (PAP), right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), cardiac index (CI), systemic vascular resistance (SVR) and rate pressure product (RPP) are reported at control, during NTG 1 μg/kg/min and during NTG 2 μg/kg/min. *p < 0.05 v corresponding control value (ANOVA)

decrease in PCWP. NTG had little effect on cardiac afterload, since SVR remained unchanged.

At sinus rhythm, NTG 1 μg/kg/min did not affect CSBF, but decreased MVO₂ (figure 5.1). However, during NTG 2 μg/kg/min, neither CSBF nor MVO₂ were different from control, despite the significant reduction in preload. The absence of a change in MVO₂ during NTG 2 μg/kg/min may however be related to the small reflex increase in sinus rate at that time. At sinus rhythm, NTG 1 μg/kg/min and NTG 2 μg/kg/min did not influence myocardial oxygen extraction percentages. These findings suggest that neither NTG 1 μg/kg/min nor NTG 2 μg/kg/min caused any direct coronary vasodilation.

3.4 NTG and metabolic coronary flow regulation

Compared to control, pacing induced changes in HR were smaller during infusion of NTG, since sinus rate increased from 65 (7) bpm at control to 69 (8) bpm during NTG 2 μg/kg/min, while pacing rate remained unchanged (94 (6) bpm, 95 (8) bpm and 95 (8) bpm at control and during NTG 1 μg/kg/min and NTG 2 μg/kg/min, respectively).

The increase in MVO₂, that was induced by pacing, was significantly attenuated by infusion of NTG (figure 5.1). The accompanying increase in CSBF was also reduced during NTG infusion, but to a lesser extent than the increases in MVO₂. Consequently, MVO₂ during pacing was significantly reduced during NTG 1
Figure 5.1: Values of coronary sinus blood flow (CSBF), myocardial oxygen consumption (MVO₂), and coronary sinus hemoglobin oxygen saturation (S₉O₂) are shown at sinus rhythm (SR), during pacing, and again at sinus rhythm (after cessation of pacing) for each of the following conditions: control, during infusion of nitroglycerin (NTG) 1 µg/kg/min, and during NTG 2 µg/kg/min. Note that values after cessation of pacing always returned to values obtained before pacing. Values are shown as mean (SEM). *p<0.05 v corresponding value at control (ANOVA).
Figure 5.2: Effect of nitroglycerin (NTG) on metabolic coronary flow regulation. Shown is the effect of small pacing induced changes in heart rate (30 beats/min above sinus rhythm) on myocardial oxygen supply (O₂ supply) and myocardial oxygen consumption (MVO₂) at control (upper panel) during administration of NTG 1 μg/kg/min (middle panel) and NTG 2 μg/kg/min (lower panel). The dashed line in the upper panel, characterizing metabolic coronary flow regulation at control, is the result of regression analysis to the data (ΔO₂ supply = 1.39 (95% CI: 1.29 - 1.49) · ΔMVO₂) and was used as reference in the middle and lower panels. The regression line (ΔO₂ supply = 1.44 (95% CI: 1.33 - 1.56) · ΔMVO₂) calculated from the data during infusion of NTG 1 μg/kg/min was not significantly different from the reference line at control. During NTG 2 μg/kg/min, the slope of the calculated regression line (ΔO₂ supply = 1.84 (95% CI: 1.63 - 2.05) · ΔMVO₂) was significantly increased compared to the reference line at control (ANOVA: p<0.01), suggesting increased metabolic coronary vasodilation during NTG 2 μg/kg/min.
EXOGENOUS NO AND CORONARY FLOW CONTROL

μg/kg/min and NTG 2 μg/kg/min, compared to MVO₂ during pacing at control, whereas CSBF during pacing remained unchanged (figure 5.1).

The individual changes in myocardial oxygen supply related to the pacing induced changes in MVO₂ during NTG 1 μg/kg/min and NTG 2 μg/kg/min, are shown in figure 5.2 (middle and lower panel, respectively). During NTG 1 μg/kg/min, metabolic regulation was characterized by a supply-demand ratio of 1.44 (95% CI: 1.33 - 1.56), which was not significantly different from the supply-demand ratio at control. However during NTG 2 μg/kg/min, this ratio was significantly increased to 1.84 (95% CI: 1.63 - 2.05; ANOVA: p < 0.01). Thus, during NTG 2 μg/kg/min, the increase in myocardial oxygen supply, related to the pacing induced change in MVO₂, was larger than at control. This was also illustrated by an increase in coronary sinus SO₂ (S_c, O₂) during pacing, from 30 (5) % at control to 34 (7) % during NTG 2 μg/kg/min (p = 0.018) (figure 5.1).

3.5 Rate of metabolic coronary flow regulation

The coronary response rates to pacing and cessation of pacing, during control, NTG 1 μg/kg/min and NTG 2 μg/kg/min, were quantified by tₜ₀-values. The individual tₜ₀ values for the dilating and constricting responses, induced by increases and decreases in HR, were averaged, since no direction sensitivity of tₜ₀ values was observed [25]. At control, the mean tₜ₀ value of the coronary response to pacing was 6.2 (0.8) s. During NTG 1 μg/kg/min and NTG 2 μg/kg/min, the tₜ₀ value of this coronary response remained unchanged, being 6.2 (1.1) s and 5.5 (1.4) s, respectively.

3.6 Lactate balance

Figure 5.3 shows the calculated values of MLU during all measurements at sinus rhythm and during pacing. Due to the pacing induced increases in CSBF, MLU tended to increase during pacing, which reached significance during administration of NTG 1 μg/kg/min (p = 0.029).

However, during the entire study period both at sinus rhythm and during pacing myocardial lactate extraction remained unchanged. Furthermore, none of the patients showed conversion from myocardial lactate extraction to production in response to the relatively modest changes in HR that were induced by pacing. In addition, we did not observe electrocardiographical signs of myocardial ischemia throughout the study period. Thus, changes in myocardial oxygen supply most likely resulted from physiological metabolic coronary flow regulation, without the effect of confounding factors brought about by myocardial ischemia.

4. Discussion

This study in patients with CAD showed that infusion of the NO donor substance NTG attenuated pacing induced increases in MVO₂ and increased the ratio of pacing induced changes in myocardial oxygen supply and MVO₂. This ratio, reflecting normal metabolic coronary flow regulation, was 1.39 (95% CI: 1.29 - 1.49) at control. During NTG 2 μg/kg/min, this ratio increased to 1.84 (95% CI: 1.63 - 2.05). This
suggests that infusion of the NO donor substance NTG at a rate of 2 μg/kg/min caused more metabolic coronary vasodilation than at control.

In the present study, metabolic coronary flow regulation was quantified by the myocardial oxygen supply-consumption ratio. Recently, Noble [24] recommended the use of this ratio as a refinement to more conventional analysis techniques used to evaluate coronary vasodilator drugs, since the effect of these drugs on coronary blood flow may also result from simultaneous drug induced changes in MVO$_2$ and normal metabolic coronary flow regulation.

Using this technique in the present study, we were able to evaluate the effect of NTG on metabolic coronary flow regulation, while accounting for the possible confounding effect of the drug on the metabolic stimulus itself (i.e. the pacing induced increase in MVO$_2$). This is particularly important, since NTG has been reported to attenuate pacing induced increases in MVO$_2$, as was shown by Ihlen et al., who studied the potential adverse effects of NTG on myocardial ischemia in patients with CAD [27].

In agreement with the study by Ihlen, we found that pacing induced increases in MVO$_2$ were attenuated by 60% during NTG 2 μg/kg/min (figure 5.1). This may partly be attributed to the 22% lesser increase in rate pressure product (i.e. the product of HR and SBP), that was found during NTG 2 μg/kg/min [28]. Since pacing rate was kept constant, the lesser increase in RPP was mainly attributable to the decrease in SBP. However, since the reduction in arterial blood pressure occurred both at sinus rhythm and during pacing, this cannot serve as an explanation for the reduced pacing-induced increase in MVO$_2$ during NTG 2 μg/kg/min. Additional explanations for the attenuated increase in MVO$_2$ during NTG infusion.
may have resided in an inhibiting effect of NTG on myocardial function [14,29], and involvement of the Gregg phenomenon.

Paulus et al. [14] showed that biconary infusion of the NO donor substance sodium nitroprusside reduced left ventricular pressure development in patients without CAD investigated for atypical chest pain. Although the mechanism behind this observation has not yet been elucidated, it might be related to recent findings by Hintze and co-workers [30-33], suggesting that NO directly reduces MVO₂ for comparable levels of cardiac work, possibly by inhibiting the mitochondrial respiration. Thus, in addition to its well established vasodilatory effect, NO may also function to modulate myocardial metabolism. This latter mechanism might theoretically explain the negative inotropic effect of NO, since if mitochondrial function is depressed by elevated NO levels, ATP synthesis needed for force development is probably reduced. Our present findings that administration of an NO donor reduces oxygen consumption more than can be explained by the confounding change in rate-pressure product indeed supports the idea that NO may impair myocardial metabolism and may extend the previous findings in dogs [30,32] and non-human primates [31] to patients with coronary artery disease.

The reduction in ABP during administration of NTG may have induced the Gregg phenomenon, i.e. the coronary-perfusion-pressure-dependency of MVO₂. However, to the best of our knowledge, the Gregg effect has only been described in animal experiments, whereas it has never been documented in humans, which makes the clinical relevance of this phenomenon questionable. In the present study, both at sinus rhythm and during pacing a similar reduction in ABP was observed after administration of NTG. Thus, if the Gregg effect had played a role, it should have influenced MVO₂ both at sinus rhythm and during pacing. However, MVO₂ at sinus rhythm was not changed by administration of NTG 2 μg/kg/min., which pleads against an important role for the Gregg effect, although we cannot rule out the possibility that the minor reflex-increase in sinus rate during NTG 2 μg/kg/min may have masked a Gregg-effect-induced decrease in MVO₂.

Whatever the cause of the attenuated increase in MVO₂, this finding demonstrates the importance of measuring MVO₂ directly, while studying the effect of a drug on metabolic coronary flow regulation. This is illustrated in figure 5.1. From the graph depicting CSBF, one might erroneously conclude that metabolic coronary vasodilation was attenuated during NTG 2 μg/kg/min, since pacing induced increases in CSBF were blunted at that time. However, the opposite is true, since changes in CSBF are dependent on, and should be related to pacing induced changes in MVO₂. If coronary flow remains unchanged in the presence of reduced myocardial oxygen demand, vasodilation has occurred. Looking at changes in both CSBF and MVO₂, it is clear that in addition to the blunted pacing-induced increase in MVO₂, more metabolic vasodilation has occurred with NTG 2 μg/kg/min. The increased supply-consumption ratio and the increase in SₐO₂ during pacing in response to NTG 2 μg/kg/min, support this finding. Therefore, conclusions regarding metabolic coronary vasodilation based on coronary blood flow values alone, should be interpreted with caution.
4.1 The role of nitric oxide in metabolic coronary vasodilation

Although the effect of NO donor substances on coronary blood flow and MVO$_2$ has been studied in conditions of myocardial ischemia [15-17,27,34], the effect on the regulation process itself has not been studied before. Kedem et al. [15] reported that exogenous NO administered as NTG 20 µg/kg i.v. improved myocardial oxygen supply-consumption ratios of partially ischemic areas of canine myocardium and they concluded that NTG improved the microregional relationship between coronary blood flow and metabolism [15,34]. However, their finding may at least partly have been the result of an effect of exogenous NO on coronary metabolic flow regulation. The present study, looking at the effect of exogenous NO on physiological metabolic coronary flow regulation (in response to submaximal changes in HR without inducing myocardial ischemia), supports this finding.

In this perspective, it is interesting that endothelium derived NO has recently been suggested as a mediator in metabolic coronary flow regulation [5]. In both dogs and humans, it was shown that inhibition of NO synthetase significantly attenuated pacing induced increases in coronary blood flow [1,3-5,11]. However, others could not confirm these findings [6,7], which might be related to the erroneous assumption that inhibiting NO does not influence the increase in MVO$_2$ [30,32].

Furthermore, it was shown that in patients with CAD metabolic coronary vasodilation was reduced [3,7,8,11], which may be explained by diminished endogenous NO activity associated with endothelial dysfunction in these patients [12,35]. NO donor substances may then act as a substitute therapy for a failing physiological mechanism, thereby partly restoring the effect of the reduced endogenous NO activity in these patients. Consequently, this may lead to more pronounced vasodilation in response to a metabolic stimulus, as was found in the present study.

Alternatively, the administration of NTG may have lead to changes in the vascular distribution of total coronary vascular resistance [36-38], shifting the predominant site of resistance in the direction of the smaller microvessels, that are most sensitive to metabolite- and pressure-mediated dilation [1,39]. If during administration of NTG, total coronary vascular resistance is mainly dominated by these smaller coronary vessels, then it is conceivable that a metabolite-induced stimulus such as pacing, induces more pronounced vasodilation, compared with the situation in which total coronary vascular resistance is dominated by the larger coronary vessels, located more upstream. Theoretically, this shift in the spatial distribution of coronary vascular resistance may explain the more pronounced metabolic coronary vasodilation in response to pacing found during administration of NTG 2 µg/kg/min, and it may pose an alternative mechanism by which NTG may influence metabolic flow control.

4.2 Limitations

One might argue whether the increased metabolic coronary vasodilation during NTG 2 µg/kg/min, resulted from NTG induced vasodilation of epicardial stenoses, shifts in the lower autoregulatory breakpoint, intracoronary shunting (coronary steal),
or collateral flow.

In our patients with CAD, potential vasodilation of coronary stenoses may have occurred in response to NTG [40]. But, this does not necessarily lead to increases in coronary blood flow, since coronary autoregulation is to compensate for the decreased pressure drop across the stenosis [41]. Only dilation of those critical stenoses that cause a pressure drop sufficient to induce \textit{maximal} post-stenotic vasodilation, will increase coronary blood flow, proportional to the increased poststenotic perfusion pressure. But, if such a stenosis had been present in our patients, then a substantial decrease in \( S_{\text{O}_2} \) would probably have occurred in response to pacing at control. (The stenosis would have limited an increase in flow, with the result that the increase in MVO$_2$ could only have been matched by an increase in myocardial oxygen extraction.) The findings that coronary blood flow did not increase with NTG infusion, and that \( S_{\text{O}_2} \) did not decrease in response to pacing, therefore suggest that vasodilation of critical coronary stenosis by NTG did not play a major role in the present study, although regional differences in myocardial oxygen extraction may have remained undetected in the coronary sinus.

In dogs, Smith and Canty [42] showed that inhibiting NO synthase increased the lower autoregulatory break point from 45 mmHg to 61 mmHg. Inversely, although speculative, NTG may reduce the autoregulatory breakpoint, which would increase coronary blood flow for pressures lower than the 'breakpoint-pressure'. However, if pacing-induced vasodilation would have lead to post-stenotic pressures lower than the 'breakpoint-pressure', this would have lead to increased myocardial oxygen extraction, which we did not observe.

The finding that NTG did not change \( S_{\text{O}_2} \) at sinus rhythm pleads against the presence of intracoronary shunting, because in case of shunting, an increase in \( S_{\text{O}_2} \) would have been expected. In addition, during infusion of NTG, none of the patients complained of angina, showed ECG changes or converted to myocardial lactate production. This is in line, with the concept that NTG, unlike other vasodilators, does not have the capacity of inducing coronary steal [13].

An increase in collateral flow induced by NTG might also explain our findings [27,43]. However, these contributions to regional coronary blood flow have shown to be limited, especially when MVO$_2$ is increased [44].

### 4.3 Effect of NTG on dynamic coronary response to pacing

In animal experiments it was shown that the dynamic coronary response to pacing is species dependent and can specifically be influenced by drugs [45,46]. To date little is known about the dynamics of coronary flow control in humans. Recently, Van Wezel et al. [25] reported that in patients with CAD, the response rate by which coronary blood flow adjusts to changes in HR was characterized by a \( t_{50} \) value of 5.2 (1.6) s. This is in agreement with the \( t_{50} \) value found at control in the present study. Our findings furthermore show that NTG did not influence the rate of the coronary response to a change in MVO$_2$. Therefore, it is unlikely that exogenous NO plays a role in the rate of coronary blood flow regulation.
5. Conclusions

NTG, a donor of exogenous NO, significantly blunted pacing induced increases in MVO$_2$. Accounting for the blunted increase in MVO$_2$ during NTG 2 µg/kg/min, it was shown that the accompanying increase in myocardial supply was relatively larger during NTG 2 µg/kg/min, than at control. This suggests that exogenous NO may increase metabolic coronary vasodilation in patients with CAD, possibly by restoring the reduced endogenous NO activity in these patients and/or by changing the vascular distribution of total coronary vascular resistance.

6. References