Prolonged Diastolic Time Fraction Protects Myocardial Perfusion When Coronary Blood Flow Is Reduced

Daphne Merkus, PhD; Fumihiko Kajiya, MD, PhD; Hans Vink, PhD; Isabelle Vergroesen, PhD; Jenny Dankelman, PhD; Masami Goto, MD, PhD; Jos A.E. Spaan, PhD

Background—Because coronary blood flow is impeded during systole, the duration of diastole is an important determinant of myocardial perfusion. The aim of this study was to show that coronary flow modulates the duration of diastole at constant heart rate.

Methods and Results—In anesthetized, open-chest dogs, diastolic time fraction (DTF) increased significantly when coronary flow was reduced by lowering perfusion pressure from 100 to 70, 55, and 40 mm Hg. On average, DTF increased from 0.47±0.04 to 0.55±0.03 after a pressure step from 100 to 40 mm Hg in control, from 0.42±0.04 to 0.47±0.04 after administration of adenosine, and from 0.46±0.07 to 0.55±0.06 after L-NMMA (mean±SD, 6 dogs for control and adenosine, 4 dogs for L-NMMA, all P<0.05). Flow normalized to its value at full dilation and pressure of 90 mm Hg (375±25 mL/min) increased during the period of reduced pressure at 40 mm Hg; control, from 0.005±63 (2 seconds after pressure step) to 0.09±0.06 (15 seconds after pressure step); with adenosine, from 0.19±0.06 to 0.22±0.06; and with L-NMMA, from 0.013±0.007 to 0.12±0.02 (all P<0.05). The increase in DTF at low pressure may be explained by a decrease in intrastitial volume at low pressure, which either decreases the preload of the myocytes or reduces the buffer capacity for ions determining repolarization, thereby causing an earlier onset of relaxation.

Conclusions—Because the largest increase in DTF occurs at pressures below the autoregulatory range when blood flow to the subendocardium is closely related to DTF, modulation of DTF by coronary blood flow can provide an important regulatory mechanism to match supply and demand of the myocardium when vasodilatory reserve is exhausted.

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Key Words: diastole ■ metabolism ■ circulation ■ perfusion ■ contractility

With progression of atherosclerosis, the increase of resistance in the diseased coronary artery is compensated by reduction of tone in resistance vessels. However, when dilatory reserve is exhausted, coronary flow will depend strongly on the relative duration of diastole. This DTF is determined by heart rate and by factors that modulate systolic duration through modulation of myocyte contraction, such as endothelin-1, angiotensin II, and nitric oxide. In earlier studies, it has been demonstrated that occlusion of a major coronary artery results within seconds in shortening of the duration of systole. However, because heart rate was not controlled, this may be explained in part by an increase in heart rate.

We hypothesized that at constant heart rate, the effect of a decrease in perfusion pressure on coronary flow could be compensated by an increase in diastolic time fraction (DTF). This would be especially beneficial for subendocardial perfusion. An increase in diastolic duration may therefore be an important mechanism for matching coronary supply and demand of oxygen by simultaneously decreasing demand and increasing supply.

Methods

Six dogs with a body weight of ~20 kg were sedated with an injection of ketamine (10 mg/kg IM) followed by an injection of sodium pentobarbital (25 mg/kg IV) for anesthesia. Depth of anesthesia was monitored by checking reflexes, and additional anesthesia was given when necessary. After tracheal intubation, dogs were ventilated with a mixture of room air and oxygen by use of a jet ventilator at a rate sufficient to maintain arterial oxygen and carbon dioxide tensions in the physiological range (pH 7.35 to 7.45, PCO 2 25 to 40 mm Hg, PO 2 >70 mm Hg). When necessary, sodium bicarbonate was given to avoid acidosis. A thin polyethylene catheter was inserted into the left jugular vein for administration of drugs. An 8F pigtail double-lumen manometer catheter (microtip catheter transducer, model SPC-784A, Millar) was inserted, with 1 of its sites of measurement placed in the left ventricle and the second in the ascending aorta. A medial sternotomy and a thoracotomy between the third and fourth ribs were performed, and the heart was...
suspended in a pericardial cradle. Two pacing wires were sewn onto the right atrial appendage. The sinus node was destroyed by injection of 40% formaldehyde, and the heart was paced at 100 to 120 bpm. After administration of an initial intravenous dose of heparin followed by continuous administration, the heart was perfused by means of a perfusion system applying a stainless steel Gregg cannula ligated into the left main coronary artery without disruption of flow. Coronary blood flow was measured with an in-line flow probe (Transonic 4F, T206). Perfusion pressure was measured with a thin fluid-filled catheter at the cannula tip or with a fluid-filled 24-gauge catheter in the first diagonal branch of the LAD. All experiments were done in accordance with the guidelines on animal experiments of our institutions.

Protocol
Coronary arterial pressure was decreased stepwise from 100 mm Hg to 70, 55, and 40 mm Hg, kept at that level for 20 to 30 seconds, and then increased back to 100 mm Hg. The interventions were repeated with a stenosis on the perfusion line and adjustment of reservoir pressure to obtain similar average coronary pressures. This protocol was repeated 1) after administration of adenosine (20 to 50 μg · kg⁻¹ · min⁻¹ IC) such that reactive hyperemia resulting from 15 seconds of occlusion disappeared and 2) after 20 minutes of administration of N⁶-monomethyl-L-arginine (L-NMMA) (4 μmol · kg⁻¹ · min⁻¹) (4 dogs) blocking nitric oxide synthesis. Reduction of nitric oxide synthesis was confirmed by comparing dilation to acetylcholine (1 μg/kg) before and after administration of L-NMMA in 3 dogs.

Determination of the Relative Duration of Diastole
Diastole was defined as the period when left ventricular (LV) pressure was ≤25% of the range between its minimal and maximal values and DTF as the quotient of the durations of diastole and the entire heartbeat.

Statistics
The influences of adenosine, L-NMMA, and coronary pressure on DTF were determined by ANOVA. If significant differences were found, a pairwise multiple comparison method (Bonferroni) and paired t tests were used to test the differences between the individual groups.

Results
Figure 1 shows a typical example of the influence of perfusion pressure on DTF. After pressure decreased stepwise from 100 to 35 mm Hg (top panel), flow decreased from 38 to −30 mL/min and then increased slowly to 12 mL/min (second and third panels). Maximal LV pressure and aortic pressure remained constant during the intervention (fourth panel). DTF (bottom panel) increased from 0.45 to 0.57 during the period of reduced pressure. The increase in DTF started 6.0 ± 0.7 beats (3 seconds) after the decrease in pressure and reached 50% of the maximal response after another 4 seconds. On average, these numbers were 3.4 ± 0.7 and 3.3 ± 0.7 seconds (mean ± SD), respectively. The increase in DTF reversed rapidly during reactive hyperemia when pressure was restored (Figure 2).

Figure 3 shows LV pressure in control and after administration of adenosine and L-NMMA in more detail. The traces immediately after a pressure step from 100 to 40 mm Hg (solid line) and 15 seconds later (dashed line) are superimposed, with the rise in LV pressure used as reference. The early phase of contraction and the rate of relaxation were comparable in both situations. However, relaxation started earlier, 15 seconds after the decrease in coronary pressure, resulting in prolonged duration of diastole in all conditions. Figure 4 depicts different interventions for 1 dog. Steady-state DTF is plotted as a function of pressure and as a function.
of flow. DTF increased with decreasing coronary pressure with or without stenosis. With adenosine, DTF is lower at all pressures, and the onset of the increase in DTF on a decrease in pressure was delayed (4.8 ± 0.7 seconds after adenosine compared with 3.4 ± 0.7 seconds in controls). The relation between pressure and DTF in the presence of L-NMMA was not different from that in controls. The reduction of DTF by adenosine is consistent with the DTF-flow relationships in the presence of tone (Figure 4, right).

Figure 5 depicts the average steady-state DTF for the different interventions as a function of flow normalized with respect to flow at full vasodilation and pressure of 90 mm Hg (average for 6 dogs in control and with adenosine, 4 dogs with L-NMMA). DTF increased with decreasing flow in a nonlinear way. At normalized flows >0.375 (150 mL/min), DTF was not influenced by changes in flow, whereas DTF is strongly related to flow below this threshold. Furthermore, the low-flow data obtained with adenosine fit within the relation obtained in controls and after administration L-NMMA.

Both DTF and flow increased with time after an initial period of ≈3 seconds, excluding capacitive effects. On average, DTF increased from 0.47±0.04 to 0.55±0.03 after a pressure step from 100 to 40 mm Hg in control, from 0.42±0.04 to 0.47±0.04 with adenosine, and from 0.46±0.07 to 0.55±0.06 with L-NMMA (all mean±SD, 6 dogs on control and adenosine, 4 dogs for L-NMMA, all P<0.05). Normalized flow also increased during the period of reduced pressure at 40 mm Hg: from 0.005±0.063 (3 seconds after pressure step) to 0.09±0.06 (15 seconds after pressure step) in controls, from 0.19±0.06 to 0.22±0.06 with adenosine, and from 0.013±0.007 to 0.12±0.02 with L-NMMA (all P<0.05). With adenosine, the increase in flow must be due to the increase in DTF. In control and after L-NMMA, vasodilation contributes to the increase in flow as well.

The relations between DTF and flow for the different conditions in 1 representative experiment are depicted in Figure 6 (see Table 1 for average data). The course of events is as follows: Flow decreases (measured after the first 3 seconds) after a drop in coronary pressure, as indicated by the vertical dashed lines in Figure 6. The data points and regression lines give the simultaneous increase of DTF and flow during the 15-second equilibration time. These relations are steeper in control than after adenosine, because of
autoregulation in the former condition. With a pressure step to 70 mm Hg, there is almost no increase in DTF; hence, the increase in flow must be due to vasodilation. At 35 mm Hg, however, the slope of the relation between DTF and flow in control is only slightly steeper than that with adenosine, indicating a relatively small contribution of vasodilation to the increase in flow at low pressure.

Eventually, the flow and DTF reach a new steady state, represented by the continuous curve in Figure 6, which is the best fit for this condition in this experiment (compare with steady-state relation between DTF and flow in Figure 4, right). Hence, because of the hyperbolic relation between DTF and flow, there is little change in DTF in the high flow range of Figures 4 (right) and 5. This interpretation is also consistent with the rather constant DTF at pressures >70 mm Hg in the presence of tone, as shown in Figure 4, left, because flow is rather independent of pressure in that pressure range.

**Discussion**

We clearly demonstrate that such shortening occurs even when heart rate is controlled. We reported our results as DTF because this parameter is closely related to subendocardial perfusion.\(^3,4\) We found that DTF is unaffected by changes in coronary flow above \(\approx 150\) mL/min, which is somewhat higher than the physiological control flow in our experiments with autoregulation intact. The increase in DTF was related to the reduction in flow below this threshold, ie, flows within or below the autoregulatory range. We further demonstrated that this flow-related phenomenon is not based on production of NO. Because increases in DTF occur mainly when vasodilatory reserve is exhausted, DTF-related increases in myocardial perfusion may benefit myocardial oxygen supply when perfusion is compromised.

**Discussion of Methods**

We defined diastole as the period when LV pressure was <25% of the range between its minimal and maximal values. Estimating DTF as the period between minimal and maximal LV dP/dt or as the period between minimal LV dP/dt and opening of the aortic valve for 1 pressure step in each dog\(^3,4,9,12\) resulted in values of DTF that were 0.11 ± 0.02 and 0.10 ± 0.03 higher than our initial estimate (both \(P<0.05\)). However, the changes in DTF on a change in pressure were similar (\(P=0.85\) and \(P=0.40\)).

In earlier studies reporting on shortening of systole, a coronary branch was occluded.\(^9,12\) These experiments demonstrate a reduced ventricular relaxation rate, which can be explained by an inhomogeneous onset of relaxation. Minimal LV dP/dt, as an index of relaxation rate, did not change during our interventions with total perfusion.

The same increase in DTF was found at different rates of pressure decrease (from stepwise to slow ramps of 3 mm Hg/s). The increase in DTF on a decrease in flow is not species dependent, because we found the same in goats.

**Possible Mechanisms of Increasing DTF**

Ischemia may occur at low perfusion pressure. Fifteen beats after the onset of underperfusion, phosphocreatine and ATP contents decrease in the subendocardium, without a change in lactate content.\(^11\) This may open the ATP-dependent K\(^+\) channels, which can result in an earlier onset of relaxation. However, glibenclamide (220 mL, 1 mg/mL) administered in similar experiments performed in goats did not influence the

**TABLE 1.** Slopes of the Relations Between DTF and Flow Normalized to Flow at 90 mm Hg at Full Vasodilation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Average Flow per Beat</th>
<th>Maximal Flow per Beat</th>
<th>Minimal Flow per Beat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Pp, 38±3 mm Hg</td>
<td>0.9±0.19</td>
<td>1.0±0.37*</td>
<td>0.39±0.2</td>
</tr>
<tr>
<td>Control Pp, 55±3 mm Hg</td>
<td>2.02±0.24</td>
<td>2.87±0.41*</td>
<td>0.2±0.08*</td>
</tr>
<tr>
<td>Adenosine Pp, 42±3 mm Hg</td>
<td>0.26±0.05†‡</td>
<td>0.08±0.11†‡</td>
<td>0.18±0.08†‡</td>
</tr>
<tr>
<td>L-NMMA Pp, 39±2 mm Hg</td>
<td>0.9±0.17</td>
<td>1.33±0.32*</td>
<td>0.41±0.17*</td>
</tr>
<tr>
<td>L-NMMA Pp, 57±2 mm Hg</td>
<td>1.87±0.38</td>
<td>2.58±0.42*</td>
<td>1.36±0.48*</td>
</tr>
</tbody>
</table>

Pp indicates perfusion pressure. Values are mean±SEM per % increase in DTF.

*Significantly different from slope average flow.
†Significantly different from control.
‡Significantly different from L-NMMA.
change in DTF during total coronary occlusion (data not shown), making a role of the ATP-dependent K⁺ channels unlikely.

Some other observations make it unlikely that DTF increased as a result of a possible shortage of oxygen: at a perfusion pressure of 100 mm Hg, DTF decreased when flow was increased by adenosine, implying that DTF can also vary in a nonischemic heart. Moreover, injections of anoxic saline in similar experiments always resulted in a decrease in DTF (14 injections in 5 dogs, typical example in Figure 7) while decreasing the oxygen content of blood. Furthermore, DTF increases only in the first 15 seconds after the pressure reduction and is stable thereafter, whereas a possible degree of ischemia will develop further in time, depending on the amount of flow reduction.

Table 2 lists a series of substances produced by endothelial cells that may influence myocyte function. From this list, only the production of nitric oxide can change fast enough to explain the increase in DTF. From similar experiments it always resulted in a decrease in DTF (14 injections in 5 dogs, typical example in Figure 7) while decreasing the oxygen content of blood. Furthermore, DTF increases only in the first 15 seconds after the pressure reduction and is stable thereafter, whereas a possible degree of ischemia will develop further in time, depending on the amount of flow reduction.

DTF changes induced by underperfusion are compatible with a dominant role of interstitial volume that may alter the contraction duration of the myocytes either through influencing the sarcomere length or through changes in extracellular concentration of ions involved in repolarization. Interstitial volume variations are to be expected with changes in flow, both in control and with adenosine, by variation of capillary pressure but also with saline infusion by decreasing plasma oncotic pressure. Also, the time course of DTF variation, being slower than intravascular volume variations, is consistent with this hypothesis.

**Interpretation of Findings**

In a heart with normal coronary regulation, local flow is adapted to match the metabolic needs of the myocardium by vasodilation. However, with a severe stenosis in the coronary arteries, the possibility for further dilation of the resistance vessels by physiological stimuli is exhausted, although further pharmacological dilatation is possible. In these circumstances, coronary flow is determined by mechanical forces, such as compression of intramyocardial vessels by the surrounding myocardium during contraction. An increase in DTF will be beneficial to myocardial perfusion because the time that intramyocardial vessels are compressed decreases. Hence, lengthening of DTF may be important at some stage of transition from normal to ischemia, resulting in delayed occurrence of ischemia. This stage may be of either short or long duration, depending on the rate of development of the disease.

With a coronary stenosis, the difference between diastolic and systolic flow becomes less, but coronary pressure becomes more pulsatile. Therefore, the relative contribution of systolic flow to total flow may increase, especially if the stenosis is compliant. Hence, there are 3 factors that might have contributed to the increase in total flow at low pressure: the increase in DTF, vasodilation, and an increased contribution of systolic flow.

The effect of DTF on flow is clear when vasomotor tone is abolished and is larger at lower flow and pressure levels. The contribution of vasodilation to the increase in flow can be estimated as the difference between the slopes of the DTF-flow relations in control and with adenosine, as discussed above in relation to Figure 6. This figure also demonstrates that in the presence of autoregulation, the effect of DTF becomes more important at lower perfusion pressure.

Because vasomotor tone changes are dominant, when present, the effect of DTF on the contribution of systolic flow to total flow can be studied only in the presence of adenosine. At constant pressure, the influence of DTF on minimal (systolic) and maximal (diastolic) flow was similar (Table 1).

| TABLE 2. Time Course of Changes in Cardioactive Substances Released by Endothelial Cells |
|---------------------------------|---------------------------------|-------------------------------|
| Factor                          | Time Course of Production/Degradation | Study                        |
| Endothelin-1                    | Onset, 4 min; maximal effect, 20 min; degradation half-time, 7 min | Mebazaa et al²               |
| Angiotensin II                  | ACE inhibition effect maximal, 8–12 min | Anning et al¹⁶               |
| Nitric oxide                    | Within heart beat                  | Pinsky et al²²               |
| Atrial natriuretic peptide      | Maximal release, 12–14 min         | Chen et al²³                 |
| Myofilament-desensitizing agent | Onset, 2 min; maximal effect, 15 min | Ramaciotti et al¹⁸           |

Figure 7. Typical example of a bolus saline infusion of 10 mL, administered to separate effects of flow (interstitial volume) and hypoxia on DTF. Because DTF decreased during injection of saline, flow, not hypoxia, is most important parameter in determination of DTF.
An increase in diastolic time, at the expense of systolic time, reduces the time for squeezing blood out of the vessels and increases the time for volume recovery, thereby keeping the intramural vessels at a lower resistance. This may affect both systolic and diastolic flow.

When discussing phasic coronary flow, one has to be aware of capacitive effects. The perfusion system did not add any significant compliance, because the in-line flow probe was connected closely to a steel cannula. Coronary arterial flow is phasic, and systolic flow can be retrograde because of the intramyocardial pump action on the intramural compliance. However, the relations between DTF and systolic and diastolic flow were measured at constant coronary pressure, minimizing these epicardial capacitive effects that could have changed the phasic flow patterns. Obviously, the phasic flow patterns measured at the level of the larger arteries is different from those of the smaller vessels and veins and may be different at the subendocardium and subepicardium. However, such differences seem not to influence the DTF–arterial flow relationship.

In the presence of a rigid stenosis, as in our case, the difference between systolic and diastolic flow is minimized. Therefore, the effects of DTF on systolic and diastolic flow are similar. It should be noted, however, that when coronary resistance changes with either DTF or vasodilation in the presence of a stenosis, this affects not only flow but also the pressure drop over the stenosis. This is the reason that DTF-flow slopes are not provided in Table 1 for the stenosis case.

Because flow and DTF are changing continuously after a pressure step, the microsphere technique is not suitable for measuring subendocardial flow. However, a 1% DTF increase by lowering heart rate increases subendocardial flow by 2.6% to 6.0% but has no effect on subepicardial flow. These findings tally well with the increase of 2.6% total flow with a 1% increase in DTF in our experiments.

Shortening of the duration of contraction may also contribute to the decreased oxygen consumption found on a decrease in coronary arterial pressure. Hence, the effect of increased DTF may affect the oxygen supply-demand ratio both by increasing supply and decreasing demand.

Conclusions

At a constant heart rate, a decrease in coronary perfusion results in an increase in DTF, which cannot be explained by release of a known cardioactive factor in response to hypoxia. The hypothesis that DTF changes in relation to changes in interstitial volume is worth pursuing. However, further studies are necessary to definitively determine the relative importance of ischemia versus change in interstitial volume. Because DTF is an important determinant of subendocardial flow, it may provide a potential protective mechanism against endocardial ischemia when coronary perfusion is impaired.

Acknowledgments

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[... omitted for brevity ...]


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