Exercise unmasks autonomic dysfunction in swine with a recent myocardial infarction

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Received 19 September 2004; received in revised form 8 December 2004; accepted 14 December 2004
Available online 7 January 2005
Time for primary review 21 days

Abstract

Objective: Severe congestive heart failure is associated with autonomic imbalance consisting of an increased sympathetic and decreased parasympathetic activity. In the present study, we investigated the influence of alterations in autonomic balance on cardiovascular function in 11 swine with left ventricular (LV) dysfunction produced by a 2- to 3-week-old myocardial infarction (MI).

Methods: Swine underwent permanent occlusion of the left circumflex coronary artery resulting in MI of the lateral LV wall. Autonomic activity was studied 2–3 weeks later using blockers of muscarinic (atropine), α-adrenergic (phentolamine) and β-adrenergic (propranolol) receptors.

Results: Under resting conditions, parasympathetic and sympathetic control of the heart and coronary circulation were similar in MI and normal swine. In contrast, during exercise of MI compared to normal swine, (i) there was a more pronounced gradual inhibition of parasympathetic control of heart rate with increasing exercise intensity; (ii) circulating catecholamines increased excessively, resulting in an increased β-adrenergic influence on heart rate, while (iii) the β-adrenergic influence on global left ventricular contractility was decreased, reflecting a blunted left ventricular β-adrenergic responsiveness. Furthermore, (iv) an α-adrenergic vasoconstrictor influence was absent in the anterior LV wall of both MI and normal swine, while (v) the β-adrenergic vasodilator influence in the coronary circulation was not different between normal and MI swine, which, in conjunction with the elevated catecholamine levels during exercise, suggests a diminished β-adrenergic responsiveness of coronary resistance vessels within remote non-infarcted myocardium in MI swine.

Conclusions: Swine with a recent MI display autonomic dysfunction, which is characterized by a more pronounced inhibition of parasympathetic influence and an exaggerated increase in sympathetic drive during exercise, as well as reduced myocardial and coronary vascular β-adrenergic responsiveness.

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Keywords: Autonomic nervous system; Coronary circulation; Remodeling; Heart failure; Ventricular function

1. Introduction

Cardiac dysfunction is accompanied by a hemodynamic defense reaction consisting of salt and water retention, peripheral vasoconstriction and cardiac stimulation, which serves to partially restore cardiac output and to increase systemic vascular resistance in order to maintain arterial pressure [1]. An integral part of this defense reaction involves alterations in autonomic balance [2], consisting of an increase in sympathetic activity [3–5] and a decrease in parasympathetic activity [6–8]. In contrast to the increased resting heart rates and catecholamine levels in patients with advanced heart failure [7,9,10], exercise-induced increments in heart rate are markedly attenuated [9,10], despite exaggerated sympathetic activation [9–13]. This blunted chronotropic response to exercise is most likely due to a blunted β-adrenoceptor responsiveness [10,14], in conjunction with a limited capacity to further withdraw parasympathetic tone during exercise [7,10].

Ischemic heart disease, in particular the loss of viable contractile cardiac tissue as a result of myocardial infarction (MI), is currently the principal cause of heart failure. Left ventricular dysfunction in swine with a 2–3-week-old MI is
characterized by a depressed global left ventricular (LV) contractility and lower stroke volume under awake resting conditions [15], whereas resting catecholamine levels are normal [15,16]. At all levels of dynamic exercise, there was an exaggerated increase in catecholamine levels, but the increase in heart rate was maintained, while the increase in global LV contractility was blunted only during strenuous exercise [15]. These findings suggest that an increase in sympathetic drive during exercise counterbalances in part the attenuated cardiac β-adrenergic receptor responsiveness [12]. Alternatively, the exaggerated increase in sympathetic activity could act to compensate for a blunted (further) withdrawal of vagal tone. In addition, we observed that the exercise-induced coronary vasodilatation in response to exercise was blunted [15], which could be due to increased α-adrenergic vasoconstriction and/or diminished β-adrenergic vasodilatation [17,18]. Consequently, in the present study, we investigated autonomic control of the heart and coronary circulation in swine with a 2–3-week-old myocardial infarction during graded treadmill exercise.

2. Materials and methods

Studies were performed in accordance with the “Guiding Principles in the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and with approval of the Animal Care Committee of the Erasmus MC Rotterdam. Thirty crossbred Landrace × Yorkshire swine of either sex (2–3 months old, 22 ± 1 kg at the time of surgery) entered the study, of which 16 swine were subjected to a myocardial infarction (MI). The data of 11 out of the 14 normal swine have been reported previously [17,18].

2.1. Surgical procedures

Swine were sedated (ketamine 20–30 mg/kg i.m.), anesthetized (thiopental 10–15 mg/kg i.v.), intubated and ventilated with a mixture of O₂ and N₂O (1:2) to which 0.2–1.0% (v/v) isoflurane was added [16–18]. Anesthesia was maintained with midazolam (2 mg/kg+1 mg/kg/h i.v.) and fentanyl (10 μg/kg/h i.v.). Under sterile conditions, swine were instrumented as previously described [17,18]. Briefly, a polyvinylchloride catheter was inserted into the aortic arch, for the measurement of mean aortic pressure and blood sampling for the determination of PO₂, PCO₂, pH, O₂-saturation and hemoglobin concentration. A high fidelity Konigsberg pressure transducer was inserted into the LV via the apex. Fluid-filled catheters were also implanted in the left atrium for pressure measurements and in the pulmonary artery for infusion of drugs. A small catheter was inserted into the anterior interventricular vein for coronary venous blood sampling [17]. A Doppler flow probe was placed around the left anterior descending coronary artery (LAD) [17]. In all animals, the proximal part of the left coronary circumflex artery (LCx) was exposed, but only in MI swine the LCx was permanently occluded with a silk suture [15,16]. Two MI swine died during surgery due to recurrent fibrillation. Electrical wires and catheters were tunnelled subcutaneously to the back, the chest was closed and animals were allowed to recover. Animals received analgesia (0.3 mg buprenorphine i.m., for 2 days) and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin i.v., for 5 days). Three MI swine died during the first week after surgery.

2.2. Experimental protocols

Studies were performed 2–3 weeks after surgery. After hemodynamic measurements (lying and standing), blood samples (lying) and rectal temperature (standing) had been obtained, swine were subjected to a four-stage exercise protocol on a motor driven treadmill (1–4 km/h). Hemodynamic variables were continuously recorded and blood samples collected during the last 60 s of each 3 min exercise stage, at a time when hemodynamics had reached a steady state. Blood samples were maintained in iced syringes until the conclusion of each exercise trial. Measurements of PO₂ (mm Hg), PCO₂ (mm Hg) and pH were then immediately performed with a blood gas analyzer (Acid–Base Laboratory Model 505, Radiometer), while O₂ saturation (%) and hemoglobin (in grams per 100 ml) were measured with a hemoximeter (OSM2, Radiometer). Following the last stage, swine were allowed to rest for 90 min, after which animals were treated with vehicle (physiologic saline i.v., 10 normal and 11 MI swine), blockade of muscarinic receptors (atropine, 30 μg/kg/min i.v., 14 normal and 8 MI swine), β-adrenoceptors (propranolol 0.5 mg/kg i.v., 14 normal and 8 MI swine) or α-adrenoceptors (phentolamine 1 mg/kg i.v., 8 normal and 9 MI swine) and underwent a second exercise trial [17,18]. Animals that had received atropine underwent a third exercise trial 90 min later, in the presence of atropine (30 μg/kg/min i.v.) and propranolol (0.5 mg/kg i.v.). Animals that had received propranolol underwent a third exercise trial 90 min later, in the presence of propranolol (0.25 mg/kg i.v.) and phentolamine (1 mg/kg i.v.). All protocols were performed on different days and in random order. All drugs were freshly prepared each day.

2.3. Data analysis

Digital recording and off-line analysis of hemodynamic data, computation of myocardial VO₂, and determination of catecholamine levels have been described in detail elsewhere [16–18]. Coronary blood flow data were normalized per gram of myocardium, using the radioactive microsphere technique [15]. Statistical analysis (Statview) of hemodynamic data was performed using analysis of variance (ANOVA) for repeated measures. Analysis of co-variance (ANCOVA) was used to analyze changes in myocardial oxygen balance. Using ANOVA and ANCOVA, separate
effects of exercise, drugs and MI as well as their interactions, were determined. Thus, the effects of drugs were first analyzed in the subgroups with and without MI, after which the groups were combined and tested for interaction between the drugs and the MI, to determine whether the effects of drugs differed between normal and MI swine. Statistical significance was accepted at P<0.05. Data are presented as mean±S.E.M.

3. Results

3.1. Effects of MI on exercise response

Under resting (lying) conditions, MI swine were characterized by a 30% lower LVdP/dt_{max} and a doubling of left atrial pressure, while heart rate was slightly elevated (10%) and arterial pressure was not different (Fig. 1). Exercise resulted in blunted increments of LVdP/dt_{max}, while the increase in heart rate was maintained. Coronary blood flow and myocardial O_2 consumption responses to exercise were not different in MI from normal swine. Arterial catecholamine concentrations were not significantly elevated in MI swine under resting conditions, but showed an exaggerated increase during exercise (Fig. 1). Similarly, coronary venous noradrenaline levels measured in subsets of seven MI and four normal swine were not different under resting conditions (231±67 vs. 303±57 pg/ml, respectively), and showed an exaggerated increase during exercise in MI (noradrenaline level 1238±501 pg/ml at 4 km/h) compared to normal swine (697±82 pg/ml at 4 km/h).

3.2. Autonomic control of cardiac function after MI

3.2.1. β-Adrenergic and muscarinic receptor (M)-mediated control

β-Blockade with propranolol produced a decrease in heart rate and LVdP/dt_{max} at rest and particularly during exercise in both normal and MI swine, while mean aortic pressure remained unaffected (Fig. 2). The increased effect of β-blockade with increasing exercise intensity is consistent with the increased levels of catecholamines. The exaggerated increases in catecholamine-levels resulted in a greater β-adrenergic influence on heart rate, as evidenced by the slightly greater reductions in heart rate in response to propranolol in the MI animals (Fig. 2). In contrast, the net β-adrenergic influence on the left ventricular myocardium appeared to be reduced as the propranolol-induced decrease in LVdP/dt_{max} was attenuated in MI compared to normal animals.

M-blockade with atropine increased heart rate in normal swine at rest, with no effect on mean aortic pressure (Fig. 2). The atropine-induced increase in heart rate waned with increasing exercise intensity, although it persisted at exercise levels up to 85% of maximum heart rate. The atropine-induced change in heart rate was similar in MI and normal swine under resting conditions, but was significantly smaller in MI swine at higher levels of exercise. Atropine increased LVdP/dt_{max} in normal swine at rest and during exercise, but while atropine produced a small increase in LVdP/dt_{max} under resting conditions in MI swine, the drug did not affect LVdP/dt_{max} during exercise in MI animals. These findings indicate a more pronounced withdrawal of parasympathetic tone during exercise in MI swine.

In the presence of atropine, the propranolol-induced decreases in heart rate and LVdP/dt_{max} were larger in normal swine as compared to administration of propranolol under control conditions (Fig. 2). The exaggerated responses of heart rate and LVdP/dt_{max} to administration of propranolol in the presence of atropine were blunted in MI compared to normal swine (Fig. 2). In the presence of propranolol, addition of atropine increased heart rate but to a

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Fig. 1. Responses to treadmill exercise of left ventricular function, systemic and coronary hemodynamics and catecholamine concentrations in 14 normal swine (open circles) and 11 MI swine (solid circles). 0S=standing, 0L=lying. *P<0.05 vs. corresponding 0L; †P<0.05 vs. corresponding measurement in normal swine. Data are mean±S.E.M.
Fig. 2. Effects of \( \beta \)-adrenoceptor blockade (propranolol 0.5 mg/kg i.v.) and/or muscarinic receptor blockade (atropine 30 \( \mu \)g/kg/min i.v.) on the response to treadmill exercise in normal swine (left panels) and MI swine (right panels). \( 0_L = \) lying, \( 0_S = \) standing. \( * P < 0.05 \) vs. corresponding saline; \( ^{1} P < 0.05 \) atropine+propranolol vs. corresponding propranolol; \( ^{2} P < 0.05 \) atropine+propranolol vs. corresponding atropine; \( ^{3} P < 0.05 \) response vs. corresponding saline was different in MI from normal swine; \( ^{4} P < 0.05 \) response vs. corresponding saline during exercise was different in MI from normal swine; \( ^{5} P < 0.05 \) response to atropine+propranolol vs. corresponding atropine was different in MI from normal swine. Underlining of the significance symbols denotes significant interaction of drug effect with exercise. MAP=mean aortic pressure, CBF=coronary blood flow, MVO\(_2\)=myocardial oxygen consumption. Data are mean±S.E.M.

Fig. 3. Effects of \( \beta \)-adrenoceptor blockade (propranolol 0.5 mg/kg i.v.) and/or \( \alpha \)-adrenoceptor blockade (phentolamine 1 mg/kg i.v.) on the response to treadmill exercise in normal swine (left panels) and MI swine (right panels). \( 0_L = \) lying, \( 0_S = \) standing. \( * P < 0.05 \) vs. corresponding saline; \( ^{1} P < 0.05 \) phentolamine+propranolol vs. corresponding propranolol; \( ^{2} P < 0.05 \) phentolamine+propranolol vs. corresponding phentolamine; \( ^{3} P < 0.05 \) response vs. corresponding saline was significantly different in MI from normal swine; \( ^{4} P < 0.05 \) response vs. corresponding saline during exercise was different in MI from normal swine; \( ^{5} P < 0.05 \) response to phentolamine+propranolol vs. corresponding phentolamine was significantly different in MI from normal swine. Underlining of the significance symbols denotes significant interaction of drug effect with exercise. MAP=mean aortic pressure, CBF=coronary blood flow, MVO\(_2\)=myocardial oxygen consumption. Data are mean±S.E.M.

lesser extent than under control conditions, whereas \( \text{LVdP/dt}_{\text{max}} \) was not affected. These responses were similar in MI and normal swine and decreased during exercise. These findings indicate that most of the atropine-induced effects were mediated through enhanced \( \beta \)-adrenoceptor activation.

3.2.2. \( \alpha \)-Adrenergic and \( \beta \)-adrenergic receptor-mediated control

\( \alpha \)-Blockade with phentolamine produced similar decreases in mean aortic pressure and increases in heart rate in normal and MI swine, whereas the increases in \( \text{LVdP/dt}_{\text{max}} \)/
3.4. Coronary venous oxygen tension

Coronary venous O2 tension (mmHg) was measured at different levels of myocardial O2 consumption (MVO2). The relation between coronary venous O2 tension and myocardial O2 consumption was linear, with a steeper slope in MI swine compared to normal swine (Fig. 4). This suggests that coronary blood flow was more dependent on myocardial O2 consumption in MI swine, likely due to a decreased coronary reserve capacity.

3.6. Discussion

The major findings of this study are that swine with a 2–3-week-old myocardial infarction display: (i) an exaggerated withdrawal of parasympathetic activity on the heart during treadmill exercise; (ii) exaggerated increases in circulating catecholamine levels during exercise that result in an increased β-adrenergic influence on heart rate; (iii) a decreased β-adrenergic influence on global left ventricular contractility, reflecting a blunted left ventricular β-adrenergic responsiveness; (iv) a lack of α-adrenergic vasoconstrictor influence in the anterior LV wall; and (v) a maintained β-adrenergic vasodilator influence in the coronary vasculature, which, in conjunction with the increased catecholamine levels during exercise, suggests that coronary vascular β-adrenergic responsiveness is decreased.

4.1. Parasympathetic control in swine with a 2–3-week-old MI

Patients with advanced heart failure are characterized by a shift in the sympathovagal balance, with an increased sympathetic activity [10,12] and a blunted parasympathetic activity that is reflected in reduced heart rate variability and reduced baroreceptor reflex sensitivity [6–8,10]. Parasympathetic tone is already reduced on the day of the infarction [19,20], but may recover over a period varying from several days [19,20] to months [21–24], except in patients who subsequently develop heart failure [25]. The degree of withdrawal of parasympathetic activity following a myocardial infarction depends on the size and location of the infarcted region [26,27], and the ensuing degree of LV dysfunction [8]. Kruger et al. [28] observed in rats that a
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maximal dose of atropine (0.5 mg/kg i.v.) produced smaller increases in heart rate compared to sham animals at 3 and 28 days, but not at 56 days after myocardial infarction of the LAD region (encompassing approximately 40% of the LV). In the present study, we observed that a maximal dose of atropine produced a similar increase in resting heart rate in swine with a 2–3-week-old MI and in normal swine, suggesting normal levels of parasympathetic activity under resting conditions. In contrast, the atropine-induced increase in LVdP/dt_max, that was observed in resting normal swine, was virtually absent in resting MI swine. The cardiovascular effects of parasympathetic activity may, at least in part, be mediated via presynaptic modulation of sympathetic activity [29]. The blunted effect of atropine on LVdP/dt_max in resting MI swine could thus be due to a selective loss of muscarinic receptor-mediated presynaptic inhibition of neuronal catecholamine release in the LV myocardium, so that in MI swine atropine exerted a reduced effect on LV catecholamine release. This is supported by the observation that, in the presence of β-adrenoceptor blockade, the effect of atropine on LVdP/dt_max was abolished in both groups of animals. The loss of parasympathetic inhibition of sympathetic activity appears to have occurred selectively in the LV myocardium, because atropine increased heart rate to a similar extent in normal and MI swine and because circulating catecholamines were not significantly elevated at rest in MI compared to normal swine.

The present study is the first to investigate the withdrawal of parasympathetic tone during exercise in animals with a recent MI. In contrast to the effects under resting conditions, the atropine-induced increase in heart rate during exercise (particularly at higher exercise levels) was blunted in MI swine, while the increase in LVdP/dt_max was abolished. These results are consistent with the concept that gradual inhibition of parasympathetic influence on the heart during exercise was more pronounced in MI compared to normal swine. Importantly, these findings suggest that after MI, at a time when parasympathetic tone under basal resting conditions is normal, a more pronounced inhibition of parasympathetic tone occurs with increasing exercise intensity. Since parasympathetic activity can presynaptically modulate sympathetic activity [29], it is likely that the greater degree of withdrawal of parasympathetic tone during exercise contributed to the exaggerated increase in sympathetic activity during exercise. This is also supported by the observation that, in the presence of propranolol, the effects of atropine were no longer different between MI and normal swine.

In resting dogs, parasympathetic activity exerts a direct vasodilator influence on coronary resistance vessels that is mediated via nitric oxide [30]. In contrast, in resting swine parasympathetic activity exerts an indirect vasoconstrictor effect on the coronary resistance vessels (which wanes at increasing exercise intensity) that is mediated via inhibition of β-adrenergic vasodilatation [17]. In contrast to the loss of the inhibitory influence of the parasympathetic system on β-adrenoceptor-mediated inotropy, we observed that its effects on the coronary circulation were maintained in MI compared to normal swine. These findings indicate that at this stage of LV dysfunction, parasympathetic control of β-adrenoceptor-mediated coronary vasodilatation is unimpaired.

4.2. Sympathetic control in exercising swine with a 2–3-week-old MI

In patients with advanced heart failure plasma noradrenaline levels are already increased under resting conditions [3,11]. These increased levels result principally from increased sympathetic nerve activity although impaired reuptake may also contribute [1]. Prolonged exposure to elevated noradrenaline levels results in desensitization and downregulation of the β-adrenergic receptors [14,31,32]. During exercise, an exaggerated increase in catecholamine levels occurs at similar absolute levels of exercise [13], which is aimed at maintaining chronotropic and inotropic responses to exercise [33].

Also in swine with LV dysfunction produced by a 2–3-week-old MI, we observed exaggerated increases in arterial catecholamine levels during treadmill exercise [15], at a time when resting catecholamine levels were still in the normal range [15,16]. However, several studies have shown that arterial levels of noradrenaline may not predict intracardiac levels of noradrenaline in the human [34], and canine heart [35], and since we observed in the porcine heart that coronary venous noradrenaline levels correspond well with myocardial interstitial levels [36], we determined coronary venous concentrations in a subset of swine. Although the number of animals was too small to reach statistical significance, the exercise-induced increase in coronary venous noradrenaline concentrations was (similar to the arterial response) exaggerated in MI compared to normal swine. The exaggerated exercise-induced increases in catecholamine levels reflect increased sympathetic activity, which acts to maintain the chronotropic and inotropic responses to acute exercise. This concept is supported by the observation that β-adrenoceptor blockade produced slightly greater decreases in the chronotropic response during exercise in MI as compared to normal swine. In contrast, β-adrenoceptor blockade in MI swine resulted in a smaller decrease in global LV contractility compared to normal swine, in particular during exercise. The latter findings are consistent with a reduced left ventricular myocardial β-adrenoceptor responsiveness.

In normal swine and dogs, β-adrenergic receptor activation contributes to coronary vasodilatation during exercise. The β-adrenergic coronary vasodilatation results in an increase in myocardial oxygen delivery that is commensurate with the increase in oxygen consumption, so that myocardial oxygen extraction and hence coronary venous O_2 tension remain constant [17,37]. In the present study, the net β-adrenergic vasodilator influence on the coronary circulation appeared to be maintained in MI compared to normal swine. However, in view of the
exaggerated increases of catecholamine levels during exercise, these findings suggest a diminished β-adrenergic responsiveness of the coronary resistance vessels. From the present study, we cannot determine which β-adrenergic receptor subtype is affected, but in view of its preferential distribution in the resistance vessels, it is likely that the principal subtype involved is the β2-adrenoceptor. This is also supported by previous observations in patients with ischemic cardiomyopathy that not only β1- but also β2-adrenoceptors are downregulated [38].

In the dog, α-adrenoceptor activation limits the exercise-induced increase in coronary blood flow, thereby necessitating an increase in myocardial O2 extraction, which leads to a decrease in coronary venous O2 tension [37,39]. In contrast, α-adrenoceptors do not contribute to regulation of coronary blood flow in normal swine during exercise [17]. In the present study, we found that administration of phentolamine had also no effect on coronary venous O2 tension of MI swine. These findings indicate that even in the presence of exaggerated increments in catecholamine levels in MI swine during exercise, α-adrenoceptors do not contribute to regulation of tone in porcine coronary resistance vessels.

4.3. Clinical relevance of the model

The present study employed an experimental model in which a myocardial infarction was produced by an abrupt coronary artery ligation in otherwise healthy swine, i.e. without atherosclerosis. In contrast, patients encountering a myocardial infarction typically suffer from atherosclerosis and chronic obstructive coronary artery disease. However, in a significant number of patients, a myocardial infarction is the first symptom of ischemic heart disease, most likely due to rupture of an unstable plaque that prior to rupture does not perturb coronary blood flow [40]. Rupture of such a plaque typically results in large transmural myocardial infarction, because of lack of preceding myocardial ischemia and hence lack of formation of coronary collaterals [40]. The porcine model used in the present study, in which a myocardial infarction was produced by an abrupt coronary artery ligation in a collateral deficient heart, likely reflects the latter clinical situation.

Another difference between our porcine model and patients encountering a myocardial infarction, is the relatively young age of our pigs (3–4 months), which could influence the responses of autonomic control mechanisms to myocardial infarction and subsequent LV remodelling. Importantly, the autonomic nervous system in pigs has already matured at the age of 3 months [41,42], indicating that the age of the animals is unlikely to have influenced our results.

5. Conclusions

The present study shows that awake swine with a 2–3-week-old myocardial infarction display autonomic dysfunc-

tion, consisting of decreased parasympathetic activity and increased sympathetic drive, which is minimal under resting conditions but becomes unmasked at increasing exercise intensities. These alterations help to maintain β-adrenergic influences on heart rate and coronary vasomotor tone during exercise. However, even at this early stage of post-MI LV dysfunction, the β-adrenergic influence on left ventricular contractility is blunted, suggesting significant loss of β-adrenergic responsiveness. The observation that β-adrenergic responsiveness is already blunted within 2–3 weeks after infarction lends further support to early initiation of β-blocker therapy after myocardial infarction.

Acknowledgments

Dr. Duncker was supported by an “Established Investigator” Stipend of the Netherlands Heart Foundation (2000T038); Dr. Merkus is the recipient of a “Post-Doctorate” Stipend of the Netherlands Heart Foundation (2000T042). The authors gratefully acknowledge Mr. Rob H. van Bremen for technical assistance.

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