Recovery of Regional Contractile Function and Oxidative Metabolism in Stunned Myocardium Induced by 1-Hour Circumflex Coronary Artery Stenosis in Chronically Instrumented Dogs

Guy R. Heyndrickx, William Wijns, Dirk Vogelaers, Yvan Degrieck, Anne Bol, Godelieve Vandeplaschke, and Jacques A. Melin

Stunned myocardium produced by 1 hour of critical coronary artery stenosis was evaluated for alteration in regional mechanical function and overall oxidative and fatty acid metabolism by positron emission tomography (PET) in chronically instrumented dogs. Twenty-seven dogs, chronically instrumented for measurements of left ventricular pressure and regional myocardial wall thickening in normal and ischemic zones, were subjected to a 1-hour period of myocardial ischemia produced by graded left circumflex coronary artery stenosis, resulting in minimal residual flow. Mean transmural myocardial flow during 1-hour coronary stenosis decreased to 0.34±0.04 ml/min per gram in the ischemic zones (normal zone transmural flow, 0.96±0.10 ml/min per gram). Systolic wall thickening in the ischemic zone was almost completely abolished (−97±4%). On reperfusion, systolic wall thickening immediately resumed but remained depressed. Progressive recovery was noted with time. At 24 hours, systolic wall thickening was still depressed (−20±6%, p < 0.01). At 1 week, wall thickening had completely recovered and was no longer significantly different from the control condition. In addition, the absence of necrosis at the site of wall thickness measurements was confirmed at autopsy in all dogs. No abnormalities were found by electron microscopy in four dogs undergoing myocardial biopsies at the time of PET studies. Dynamic PET studies using [1-13C]acetate tracer (performed at 6 hours, 1 week, and 2 weeks after reperfusion) and [1-13C]palmitic acid tracer (performed at 6 hours, 12 hours, 24 hours, 1 week, and 2 weeks after reperfusion) allowed the computation of regional tissue time–activity curves in different regions of interest at different times during follow-up. Despite full reperfusion, abnormal [1-13C]acetate and [1-13C]palmitic acid kinetics were observed in the posterior segments, previously subjected to ischemia, as evidenced by a significant decrease in the slope of the early 13C clearance curve component. Repeat PET studies revealed progressive normalization of overall oxidative metabolism and fatty acid metabolism, which paralleled the time course of recovery of mechanical function. Thus, myocardial ischemia, produced by 1-hour coronary artery stenosis, followed by full reperfusion is associated with a prolonged period of postischemic mechanical and metabolic dysfunction. This transient reduction in oxygen delivery induced a prolonged impairment in fatty acid beta-oxidation as well as a reduction in overall oxidative metabolism despite full reoxygenation. A similar time course for recovery of function and metabolism was observed. (Circulation Research 1993;72:901–913)

Key Words • positron emission tomography • ischemia • [1-13C]acetate • [1-13C]palmitic acid • wall thickening

Since its first observation in 1975, it is now well established that a brief episode of myocardial ischemia produced by temporary coronary artery occlusion of 5- and 15-minute duration in conscious dogs is followed by prolonged postischemic left ventricular (LV) dysfunction.1,2 On full reperfusion, complete recovery of mechanical function is delayed for several hours. This state of postischemic dysfunction was later called “stunned myocardium.”3 Although the basic pathophysiological mechanisms responsible for this delayed functional recovery are yet to be unraveled, a number of potential causes have been identified. Although the initial search for an explanation was focused on the ischemic damage produced by the deprivation of oxygen and the accumulation of toxic metabolites, attention was later shifted to the reperfusion phase as a potential source for additional damage induced by the generation of oxygen free radicals.

From the Laboratory for Normal and Pathological Physiology (D.V.), University of Gent (Belgium); the Laboratory for Positron Emission Tomography (W.W., A.B., J.A.M.), University of Louvain, Brussels; and the Cardiovascular Center (G.R.H., Y.D.), O.L.V.-Ziekenhuis, Aalst, Belgium.


Supported by grants from FGWO (No. 3.0085.89) and FRSM (No. 3.4522.89). W.W. is supported by the C. and D. Damman Foundation. G.V. is from Janssen Research Foundation, Beerse, Belgium.

Address for correspondence: G.R. Heyndrickx, MD, Cardiovascular Center O.L.V.-Ziekenhuis, Moorselbaan 164, B-9300 Aalst, Belgium.

Received February 18, 1992; accepted December 10, 1992.
and/or cellular calcium overload, at least in some models or species.4,5 Altered calcium homeostasis and decreased calcium sensitivity at the myofibril level have been suggested as possible causes for stunning.6,7 Injury to the contractile proteins, the extracellular collagen matrix, or damage to the sympathetic cardiac nerves may also contribute in part to the mechanical impairment associated with stunning.8,9 Yet, stunned myocardium remains able to respond to exogenous and endogenous catecholamines as well as to increase in extracellular calcium, disclosing a potential inotropic reserve albeit with a blunted maximal response.10-12

Although the contractile abnormalities observed in the myocardium during and after ischemia are well defined, the metabolic alterations underlying the postischemic dysfunction in stunned myocardium have revealed conflicting data. It is established that both the overall oxygen consumption and the oxidation of fatty acids, the preferred substrates under aerobic conditions, are decreased during the initial phase of ischemia, whereas glycolysis accounts for the residual production of high-energy phosphates.13 In contrast, the pattern of substrate preference displayed by the reperfused stunned myocardium is less well defined. In isolated working rat hearts, Lopaschuk et al.14 demonstrated that fatty acids remained the preferred substrate in reperfused posts ischemic tissue. In the pig model, Liedtke et al.15 similarly found that the oxidation of labeled fatty acids was not depressed immediately after reperfusion. At variance with these results, studies using positron emission tomography (PET) and radiolabeled tracers such as [2-18F]fluorodeoxyglucose and [1-13C]palmitic acid (CPA) have consistently revealed that the later phase of reperfusion is associated with increased glucose uptake and decreased fatty acid oxidation, both in acute16 and chronic17,18 experiments, fueling the hypothesis of a shift in substrate utilization.

In addition, the relation between oxygen consumption and decreased contractile function in stunned myocardium is still a matter of debate. Depressed oxygen consumption along with depressed regional function has been observed early and late after reperfusion.19-22 However, others have reported normal23 or even increased24 oxygen consumption in posts ischemic myocardium. Little or no information regarding the persistence of metabolic disturbances after reperfusion or regarding the rate of recovery of metabolic derangements is currently available. In view of the lack of consistent information regarding the changes in oxidative metabolism in stunned myocardium, the aim of the present study was 1) to further characterize the abnormalities in overall myocardial oxidative metabolism as well as in oxidation of fatty acids after reversible ischemia and 2) to study the time course of recovery of these metabolic derangements in relation to normalization of contractile function. An important feature of this study is the combined analysis of regional contractile function and regional oxidative metabolic activity. In contrast to previous work in which stunned myocardium was studied in open-chest anesthetized animal preparations, with ischemia being induced either by a single or by repeated episodes of coronary occlusions lasting 5 or 15 minutes,10-12,19,24 we have chosen a clinically more relevant model of stunning, i.e., a severe coronary artery stenosis with minimal residual flow, allowing for maintained washout of metabolites during ischemia. Matsuoka et al.25 have previously shown that coronary stenosis also induced myocardial stunning. It was deemed important to conduct these present experiments in chronically instrumented conscious animals to avoid the profound depressant effects on coronary circulation and myocardial contractility as well as on reflex cardiac adaptation to ischemia resulting from the anesthetics and the acute surgical trauma26 but, more importantly, to allow for long-term follow-up recordings of regional function as well as repeat PET studies.

**Materials and Methods**

**Chronically Instrumented Animals**

Twenty-seven mongrel dogs weighing between 22 and 34 kg underwent surgical instrumentation for subsequent hemodynamic monitoring during the experimental protocol. Under general anesthesia induced with pentobarbital (30 mg/kg i.v.), a left thoracotomy was performed through the fifth intercostal space using sterile surgical techniques. The pericardium was incised, and the heart was exposed. The proximal left circumflex coronary artery was carefully dissected free 1–2 cm from its origin to accept a Doppler ultrasonic flow probe and a hydraulic cuff occluder. The artery was then briefly occluded to delineate the ischemic area. Pairs of ultrasonic crystals for measurement of wall thickness were implanted across the LV free wall in the center of the area intended to become ischemic, as well as in a remote normal zone in the left anterior descending coronary artery distribution. A miniature solid-state pressure transducer (Konigsberg Instruments, Inc., Pasadena, Calif.) was implanted in the LV cavity through an apical stab incision. Heparin-filled silastic catheters (Dow Corning Co.) were implanted in the ascending aorta, pulmonary artery, and left atrium. All transducer wires and catheters were tunneled subcutaneously to the dorsal neck surface, exteriorized, and attached to the skin. All animals were routinely placed on postoperative antibiotic therapy for 10 days. A minimal interval of 2 weeks was allowed for the dogs to recover from this surgical procedure. Four dogs were instrumented with only a Doppler flow probe, a cuff occluder around the left circumflex coronary artery, and one pair of ultrasonic crystals in the posterolateral zone. In these animals, myocardial biopsies were taken in normal and stunned zones 6 hours after reperfusion for morphological analysis. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the University and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (Department of Health, Education, and Welfare publication No. [NIH] 78-23, revised 1978).

**Functional and Hemodynamic Measurements**

LV pressure was measured with the implanted miniature pressure gauge, which was calibrated in vitro as well as in vivo during the experiments against systolic arterial pressure and diastolic left atrial pressure, sampled through the fluid-filled catheters, and measured with Statham P23 ID strain-gauge manometers (Statham Instruments, Oxnard, Calif.). Regional myo-
cardial function was measured with an ultrasonic transit time dimension gauge previously described in detail.\textsuperscript{27} The instrument measures the transit time of acoustic signals traveling at a sonic velocity of $1.58 \times 10^4$ mm/sec between two intramyocardial crystals. The drift of this instrument is minimal (less than 0.01 mm in 6 hours) and was effectively eliminated by repeated calibration throughout the experiment. The LV pressure derivative (dP/dt) signal was used for timing of the cardiac cycle. End-ventricular diastole was defined as the beginning of the rise of positive LV dP/dt, and end-ventricular systole was defined 20 msec before peak negative LV dP/dt. Systolic wall thickening was defined as the difference between peak systolic and end-diastolic wall thickness. The radioactive microsphere technique was used to measure regional myocardial blood flow as previously described by Domenech et al.\textsuperscript{28} In this study, $15 \pm 1 \mu m$ microspheres labeled with $^{85}$Sr, $^{11}$Sn, and $^{99}$Co were injected at different times in random order (New England Nuclear, Boston). The microspheres were suspended in 0.01% Tween 80 solution (10% dextran), agitated, and placed in an ultrasonic bath for at least 30 minutes before injection. One to 2 million microspheres suspended in 10% dextran were injected through the catheter implanted in the left atrium for determination of blood flow. An arterial reference sample, initiated 15 seconds before microsphere injection and continued for 90 seconds after completion of the injection, was withdrawn at a speed of 7.75 ml/min from the catheter implanted in the aorta. At the end of the experiment, the dogs were killed with a lethal dose of sodium pentobarbital (50 mg/kg). The heart was sliced into rings perpendicular to its long axis. The absence of infarction was confirmed by visual inspection. Samples of myocardial tissue from the LV free wall in the immediate vicinity of the implanted crystals were subdivided into three equal transmural layers from epicardium to endocardium, weighed, and placed in a gamma counter (model S 85, Canberra Industries, Inc., Meriden, Conn.) with appropriately selected energy windows. Counts were corrected for background and crossover and compared with the reference blood sample to obtain flow expressed in milliliters per minute per gram of tissue. Endocardial/epicardial blood flow ratios were obtained by dividing subendocardial blood flow by subepicardial blood flow.

**Metabolic Measurements**

Regional myocardial substrate metabolism was studied with PET using an ECAT III (CTI, Knoxville, Tenn.) one-ring device, the characteristics and performance of which have been previously described.\textsuperscript{29} The collimator aperture was set at 30 mm, resulting in a slice thickness of 15 mm at full-width half maximum. Daily calibration of the tomograph versus a well counter was performed by measuring a uniform cylindrical phantom (diameter, 20 cm) filled with a solution of germanium ($^{68}$Ge). The animals were trained to rest quietly in the tomograph. During PET studies, the dogs were positioned in the tomograph so that the image plane transversed the LV nearly perpendicular to its long axis, at the level of ultrasonic crystal implantation. Accurate positioning was guided by prior external marking of the radiopaque crystals using fluoroscopy. PET examination was performed during light sedation obtained by a continuous infusion of a fluor analogue of etomidate ($n=10$) or propionylpromazine ($n=13$) to avoid movement artifacts during the prolonged period of data acquisition. After acquisition of the transmission images needed for attenuation correction, emission data were acquired after each tracer injection with a stationary ring in a nongated mode. Images were reconstructed with a Hann filter (0.5 cutoff), giving an in-plane resolution of 8 mm at 0.61-0.77 mm\(^2\). Tracers were injected intravenously with a pump for 30 seconds, typically 5-10 mCi of either CPA or $[1^{-1}$C]acetate (AC). Serial imaging was begun with tracer administration: initially, 30-second images were acquired six times, followed by 60-second images five times, 120-second images six times, 300-second images three times, and 600-second images two times, depending on residual activity. The interval between studies was at least five to six half-lives after injection of CPA or AC to allow for $^{13}$C activity from the previous injection to decay by more than 98% ($^{13}$C has a physical half-life of 20.4 minutes).

The PET data analysis was as follows. Throughout each cross-sectional image, twelve regions of interest ($5-7 \text{ mm}^2$) were assigned to the LV myocardium, three each in the septum, the anterior, the lateral, and the posterior free wall. An additional region of interest was assigned to the center of the LV blood pool. Myocardial and blood pool radioactivity was averaged within each region of interest, and time–activity curves were constructed from the serial images and corrected for physical decay. To recover true indicator tissue concentrations (counts per pixel per second), these data need to be corrected for cross contamination of activity between myocardium and blood as well as for the partial volume effect. The geometric method described earlier by Henze et al\textsuperscript{30} was modified by means of a Monte-Carlo simulation technique, which in addition accounts for dead-time losses observed at high-count rates early after tracer injection.\textsuperscript{31,32} Data analysis for CPA and AC studies depends on the known relation between the clearance of $^{13}$C activity and fatty acid metabolism or oxygen consumption, respectively. As shown previously,\textsuperscript{21,33-38} $^{13}$C activity after CPA or AC injection is cleared from the myocardium in a biexponential pattern. This clearance pattern suggests that both tracers are distributed in tissue between at least two pools that differ markedly in turnover rate. For CPA studies, this was shown to be consistent with the metabolic fate of fatty acids in myocardium: a fraction immediately enters oxidative pathways, including beta-oxidation and the citric acid cycle, while the remainder becomes trapped in the endogenous lipid pool as polar and nonpolar lipids.\textsuperscript{33,34,36} For AC studies, the early rapid-clearance phase was shown to represent oxidation via the tricarboxylic acid cycle and correlates linearly with the regional myocardial oxygen consumption.\textsuperscript{21,37,38} Thus, the corrected segmental time–activity curves were analyzed with a multiexponential least-squares fitting routine, and slope values for the early rapid clearance phases were obtained from CPA and AC kinetics. The best fits were obtained by fitting the data with a single exponential plus a constant, and this slope value was consistently used in all studies. Segment realignment was performed...
so that for all dogs the segment with the smallest slope value did coincide.

**Experimental Protocol**

Experiments were performed 2–3 weeks after surgery, when the animals had fully recovered and no signs of infection were present. After control recording of all hemodynamic parameters, progressive inflation of the hydraulic cuff occluder was initiated. The degree of stenosis was arbitrarily set to produce severe hypokinesia or akinesia (no difference between end-systolic and end-diastolic wall thickness) and was maintained in this manner for 1 hour. Complete coronary occlusion, usually resulting in paradoxical bulging, was avoided by monitoring the Doppler flow signal. The degree of stenosis was adjusted, if needed, to maintain a continued loss of systolic wall thickening or to avoid total occlusion. The coronary stenosis was completely released after 1 hour. Myocardial function was monitored continuously during coronary artery stenosis and for the first 3 hours after reperfusion. Monitoring was then interrupted to allow the dog to be transferred to the PET facility. Hemodynamic parameters were measured again during PET imaging studies and at 1, 2, and 4 days and weekly, up to 2 weeks after reperfusion. Serial blood samples (5 ml) were withdrawn over a 24-hour period. The samples were taken at 30-minute intervals for the first hour, then every hour for 8 hours, every 2 hours for the next 8 hours, and every 4 hours for the final 8 hours. Creatine kinase in plasma was assayed spectrophotometrically as described by Rosalki, and the normal values in our laboratory range from 36 to 170 IU/l. Studies with PET were performed at ±6 hours (18 dogs), ±12 hours (four dogs), 24 hours (four dogs), 1 week (eight dogs), and 2 weeks (four dogs) after reperfusion. Whenever possible, dual tracer imaging (CPA followed by AC) was obtained.

Microspheres were injected 45 minutes after the coronary artery stenosis was initiated and again after reperfusion, at the time of PET studies at 6 hours (11 dogs), 12 hours (four dogs), 24 hours (six dogs), and 1 week (six dogs). After 3 weeks, the animals were killed with an overdose of sodium pentobarbital, and the heart was removed. Patency of the coronary artery was verified by visual inspection of the dissected vessel. Myocardial samples were taken from the normal and postischemic areas and prepared for microscopy analysis and histological examination. In four dogs with minimal instrumentation, myocardial biopsies were taken ±6 hours after reperfusion for detailed morphological examination using electron microscopy.

**Pathology**

In four dogs, tissue samples were taken from the anterior and posterior walls of the LV at the site of implantation of ultrasonic crystals. These samples were fixed in 5% formalin, embedded in parafin, sliced into 5-μm sections, and stained with hematoxylin-eosin and Masson’s trichrome to demonstrate collagen. Light microscopy was used to assess the general features of infarcted myocardium. All sections were coded so that the pathologist was unaware of the region from which the samples were taken.

Transmural biopsies (Tru-cut biopsy needle, Travel-nol Laboratories) for ultrastructural examination were obtained in previously ischemic and nonischemic myocardium from four dogs. The myocardial biopsies were subdivided into subendocardial, intramural, and subepicardial sections and immersed in the fixative for morphological assessment as well as for calcium localization. The fixative was prepared by adding 12 ml of 25% (vol/vol) glutaraldehyde solution to 100 ml phosphate buffer containing 90 mM (wt/vol) KH2PO4 and 1.4% (wt/vol) sucrose, adjusted to pH 7.4 with 1N KOH. For morphology, samples were rinsed overnight in the phosphate buffer to which 7.5% sucrose was added and postfixed in 2% OsO4 buffered to pH 7.4 with 0.05 M veronal acetate for 1 hour at 4°C. Rinsing was carried out in veronal acetate buffer, pH 7.4, supplemented with 7% sucrose for 5 minutes at 4°C. Tissue was impregnated in 0.5% uranyl acetate rinsed in veronal acetate buffer, pH 5.2.

For calcium localization purposes, the combined phosphate-pyroantimonate method was used, allowing visualization of acidic phospholipid-bound calcium by formation of an insoluble and stable complex of inorganic phosphate, calcium, and acidic phospholipids. After fixation, samples were rinsed in potassium phosphate buffer containing 7.5% sucrose, and 100-μm-thick sections were prepared with a Vibratome (Lancer, series 1000). After postfixation with a mixture of 1% OsO4 and 2% potassium pyroantimonate in 0.01M acetic acid, samples were dehydrated and embedded in Epon. Semithin sections were cut transversely through the LV wall. Sections were stained with toluidine blue. Sections examined, and criteria used for irreversible damage were intracellular clarifications, vacuolization, absence of darkly stained mitochondria, contraction band necrosis, and nuclear pyknosis. Ultrathin sections were examined either unstained or briefly counterstained with uranium acetate and lead citrate.

**Statistical Analysis**

Hemodynamic and functional data were recorded on a multichannel tape recorder and played back on a multichannel direct-writing oscillograph at a paper speed of 100 mm/sec. The analog signals were digitized and stored on an HP 1000 computer (Hewlett-Packard Co., Palo Alto, Calif.) for processing and statistical analysis (sas 6.03, SAS Institute Inc., Cary, N.C.). Mean ± SEM values were calculated for all parameters. Significant differences between responses were first tested by the analysis of variance (null hypothesis). Differences between responses versus control were analyzed by the paired Student’s t test. Since more than one comparison was made, a correction for repeated measurements was introduced by dividing the level of significance (0.05) by the number of comparisons made.

**Results**

**Effects of Coronary Artery Stenosis**

Circumflex coronary artery blood flow (Doppler probe). Coronary artery stenosis was adjusted to abolish systolic wall thickening. This was obtained by decreasing mean circumflex coronary blood flow measured by Doppler probe from 45±4 to 16±2 ml/min (p<0.01).

Regional myocardial blood flow (microspheres). As shown in Table 1, coronary artery stenosis resulted in a
significant decrease in subendocardial blood flow in the ischemic zones at the site of wall thickness measurements: 0.20±0.03 ml/min per gram compared with normal zone endocardial flow of 1.18±0.08 ml/min per gram (p<0.01). In four dogs in which biopsies were taken, the decrease in subendocardial flow was similar: 0.27±0.04 ml/min per gram.

Subepicardial flow in ischemic zones decreased less than subendocardial flow, resulting in a significant decrease in endocardial/epicardial flow ratio from 1.47±0.12 to 0.41±0.11 (p<0.01).

Global LV function. Overall LV function, measured at 45 minutes during coronary artery stenosis, was only minimally affected, with a slight but significant increase in heart rate and a decrease in LV +dP/dt but no change in arterial or LV pressures (Table 2).

Regional myocardial function (Figure 1).

Nonischemic zone wall thickening. There were no significant changes in wall thickening at any time during coronary artery stenosis except for a small increase in percent systolic wall thickening (Table 3).

Ischemic zone wall thickening. During coronary artery stenosis, systolic wall thickening in the ischemic area decreased by 97±4% of the control value. As stated in “Materials and Methods,” coronary stenosis was arbitrarily set and maintained to produce severe hypokinesia or akinesia but not dyskinesia. End-diastolic wall thickening decreased slightly (Table 3).

Ventricular arrhythmias. During the 1-hour coronary artery stenosis period, no ventricular arrhythmias were observed.

Effects of Coronary Artery Reperfusion

Circumflex coronary artery blood flow (Doppler probe). On release of the coronary stenosis, mean circumflex coronary blood flow increased by 316±28%, exhibiting a marked and prolonged hyperemic response. This hyperemic response lasted for 18±6 minutes. Measurements during late follow-up were not different from the initial control value.

Regional myocardial blood flow (microspheres). Subendocardial blood flow values in the stunned zones measured at the time of PET studies 6 hours after reperfusion and again at 12 hours, 24 hours, and 1 week were 1.25±0.09, 1.27±0.20, 1.18±0.17, and 1.13±0.22 ml/min per gram, respectively (p=NS).

Subepicardial blood flow values in the stunned zones at 6 hours, 24 hours, and 1 week after reperfusion were 0.96±0.08, 1.15±0.18, 0.97±0.10, and 0.91±0.14 ml/min per gram, respectively (p=NS).

Global LV function. On reperfusion, little change in global LV function was noted. Two weeks after reperfusion, no hemodynamic parameter was significantly different from baseline value (Table 2).

Regional myocardial function (Figure 1).

Nonischemic zone wall thickening. There were no significant changes in wall thickening at any time after reperfusion.

Ischemic zone wall thickening. Reperfusion was characterized by an immediate rebound in systolic wall thickening coinciding with the reactive hyperemic response. After the initial rebound, thickening remained

Table 1. Myocardial Blood Flow Distribution 45 Minutes After Coronary Artery Stenosis, at the Time of Positron Emission Tomography, and After 1 Week of Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>45-Minute CAS</th>
<th>CAR+PET</th>
<th>CAR+1 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi flow (ml/min per gram)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic zone</td>
<td>0.49±0.06</td>
<td>0.96±0.08</td>
<td>0.91±0.14</td>
</tr>
<tr>
<td>Normal zone</td>
<td>0.85±0.09</td>
<td>1.03±0.08</td>
<td>0.82±0.08</td>
</tr>
<tr>
<td>Endo/Epi ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic zone</td>
<td>0.41±0.11</td>
<td>1.31±0.07</td>
<td>1.21±0.07</td>
</tr>
<tr>
<td>Normal zone</td>
<td>1.47±0.12</td>
<td>1.31±0.14</td>
<td>1.36±0.11</td>
</tr>
<tr>
<td>Transmural flow (ml/min per gram)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic zone</td>
<td>0.34±0.04</td>
<td>0.96±0.04</td>
<td>1.08±0.17</td>
</tr>
<tr>
<td>Normal zone</td>
<td>0.96±0.10</td>
<td>1.12±0.08</td>
<td>1.01±0.13</td>
</tr>
</tbody>
</table>

CAS, coronary artery stenosis; CAR, coronary artery reperfusion; PET, positron emission tomography; Endo, endocardial; Epi, epicardial. Values are mean±SEM.
*p<0.01 vs. baseline values.

Table 2. Effects of Coronary Artery Stenosis and Reperfusion on Global Left Ventricular Function

<table>
<thead>
<tr>
<th></th>
<th>Pre-CAS control</th>
<th>1-Hour CAS</th>
<th>CAR+PET</th>
<th>CAR+2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>87±8</td>
<td>100±8*</td>
<td>88±8</td>
<td>83±9</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>99±5</td>
<td>104±4</td>
<td>93±3</td>
<td>98±5</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>128±8</td>
<td>126±5</td>
<td>125±5</td>
<td>132±8</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>9±1</td>
<td>10±1</td>
<td>9±1</td>
<td>10±1</td>
</tr>
<tr>
<td>LV +dP/dt (mm Hg/sec)</td>
<td>3,174±269</td>
<td>2,883±177*</td>
<td>2,987±282</td>
<td>3,180±270</td>
</tr>
</tbody>
</table>

CAS, coronary artery stenosis; CAR, coronary artery reperfusion; PET, positron emission tomography; bpm, beats per minute; LV, left ventricular. Values are mean±SEM.
*p<0.01 vs. pre-CAS control.
significantly depressed (1.10±0.25 mm) at 6 hours. Progressive recovery of systolic thickening was noticed through repetitive measurements at 24 hours, 48 hours, 1 week, and 2 weeks: 1.47±0.16, 1.55±0.14, 1.76±0.16, and 1.92±0.21 mm, respectively (baseline, 1.91±0.15 mm).

Ventricular arrhythmias. On reperfusion, none of the animals developed ventricular fibrillation. In 16 dogs, premature beats and episodes of idioventricular rhythm were temporarily observed between 12 and 24 hours after reperfusion, when function recordings were made.

PET Results

Myocardial CPA and AC kinetics. Myocardial images obtained in one dog 6 hours after reperfusion are shown in Figure 2. The initial uptake of both tracers is symmetrical, but thereafter, the 14C clearance is delayed in the postischemic lateral and posterior walls.

Representative regional time–activity curves derived from PET images are shown in Figure 3. The clearance slope of both tracers is decreased in the stunned zones.

**Table 3.** Effects of Coronary Artery Stenosis and Reperfusion on Ischemic Zone and Normal Zone Function

<table>
<thead>
<tr>
<th></th>
<th>Pre-CAS control</th>
<th>1-Hour CAS</th>
<th>CAR+PET</th>
<th>CAR+2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>End-diastolic wall thickness (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic zone (n=7)</td>
<td>9.70±0.62</td>
<td>9.46±0.64*</td>
<td>9.79±0.64</td>
<td>9.65±0.87</td>
</tr>
<tr>
<td>Normal zone (n=4)</td>
<td>8.36±0.74</td>
<td>8.20±0.73</td>
<td>8.07±0.61</td>
<td>7.73±0.75</td>
</tr>
<tr>
<td><strong>End-systolic wall thickness (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic zone (n=7)</td>
<td>11.61±0.64</td>
<td>9.52±0.60*</td>
<td>11.26±0.70</td>
<td>11.57±0.75</td>
</tr>
<tr>
<td>Normal zone (n=4)</td>
<td>10.26±1.17</td>
<td>10.29±1.07</td>
<td>9.76±0.83</td>
<td>9.38±0.90</td>
</tr>
<tr>
<td><strong>Systolic wall thickening (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic zone (n=7)</td>
<td>1.91±0.15</td>
<td>0.06±0.07*</td>
<td>1.47±0.16*</td>
<td>1.92±0.21</td>
</tr>
<tr>
<td>Normal zone (n=4)</td>
<td>1.90±0.57</td>
<td>2.09±0.47</td>
<td>1.69±0.31</td>
<td>1.65±0.15</td>
</tr>
<tr>
<td><strong>Systolic wall thickening (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic zone (n=7)</td>
<td>20.07±1.93</td>
<td>0.82±0.69*</td>
<td>15.16±1.60*</td>
<td>20.64±2.81</td>
</tr>
<tr>
<td>Normal zone (n=4)</td>
<td>22.06±5.42</td>
<td>25.15±4.44*</td>
<td>20.89±2.89</td>
<td>21.31±1.15</td>
</tr>
</tbody>
</table>

CAS, coronary artery stenosis; CAR, coronary artery reperfusion; PET, positron emission tomography. Values are mean±SEM.

*p<0.01 vs. pre-CAS control.
FATTY ACID METABOLISM. $^{11}$C clearance after CPA injection was calculated from 12 regions of interest selected from the tomographic slice. The rate constant (slope, min$^{-1}$) for the early phase was as follows: in the three lateral regions, $0.15\pm0.01$, $0.14\pm0.01$, and $0.14\pm0.01$; in the three posterior regions, $0.12\pm0.01$, $0.10\pm0.01$, and $0.12\pm0.01$; in the three septal regions, $0.14\pm0.01$, $0.15\pm0.01$, and $0.15\pm0.01$; and in the three anterior regions, $0.16\pm0.01$, $0.15\pm0.01$, and $0.16\pm0.01$. These values indicate that the lowest slope value, reflecting the slower oxidation of the fatty acids, was recorded in the stunned myocardium. These values were significantly different when compared with the nonischemic anterior zone ($p<0.02$) (Figure 4).

OVERALL OXIDATIVE METABOLISM. The same 12 regions of interest of the tomographic slice were analyzed for the slope value after AC tracer administration. The rate constant (slope) for the rapid phase was as follows: in the three lateral regions, $0.21\pm0.02$, $0.19\pm0.02$, and $0.21\pm0.03$; in the three posterior regions, $0.17\pm0.01$, $0.12\pm0.01$, and $0.20\pm0.02$; in the three septal regions, $0.22\pm0.02$, $0.19\pm0.02$, and $0.23\pm0.02$; and in the anterior regions, $0.21\pm0.02$, $0.22\pm0.02$, and $0.20\pm0.02$. These values indicate a significantly lower slope in the center of the postischemic zone, which was significantly different when compared with the nonischemic anterior zone ($p<0.02$) (Figure 4).

Repeated PET studies during reperfusion. Changes in the early-phase slope of CPA and AC clearance are compared in both the normal zone and postischemic zone. The data are expressed as the normal zone/stunned zone ratio to account for the large individual variations in basal fatty acid oxidation, occurring from dog to dog and from day to day, that is due to variable substrate availability.

Normal zone/stunned zone ratios of CPA slope at ±6 hours, 12 hours, 24 hours, 1 week, and 2 weeks were 1.48±0.06, 1.28±0.08, 1.11±0.08, 1.05±0.06, and 1.01±0.03, respectively, indicating a progressive normalization over time of fatty acid oxidation in the stunned myocardium (Table 4).

Normal zone/stunned zone ratios of AC slope at ±6 hours, 1 week, and 2 weeks were 1.36±0.12, 1.07±0.10, and 1.00±0.31, respectively, indicating normalization of overall oxidative metabolism over time (Table 5 and Figure 5).

Biochemical Measurements

After reperfusion was initiated, creatine kinase levels in plasma increased gradually from 53±9 IU/l to a peak.
of 187±30 IU/l at 4 hours. In two animals, serial plasma enzyme activity was measured before the experimental protocol, and no significant changes were observed over a 24-hour period.

**Pathology**

Coronary artery patency was demonstrated at autopsy in all dogs. Macroscopic evidence for myocardial infarction was absent in all but two dogs, in which small foci of necrosis were found at the tip of the posterior papillary muscle. No evidence of necrosis or scar tissue was found in the free wall of the LV underlying the ultrasonic crystals.

In nonischemic myocardium, subcellular structures by electron microscopy were well preserved. The glycocalyx was closely apposed to the sarcolemma, with the latter surrounding the cell without discontinuities. Mitochondria were abundant and regularly arranged between the sarcomeres, and they contained many osmiophilic granules, which are typical for well-oxygenated cells. The nucleus contained evenly dispersed chromatin. Glycogen granules were abundantly present. Calcium deposits, clearly visible as 20-nm particles, were confined to the leaflet of the sarcolemma, the T tubules, and intercalated disks. At the gap junction, the deposits were clearly visible in pairs.

Single deposits were sometimes observed in the mitochondria, whereas all other organelles were completely devoid of precipitate. After 1 hour of stenosis followed by 6 hours of reperfusion, ultrastructural changes in the ischemic zone appeared minor. Apart from the observation that in two dogs subendocardial mitochondria contained less or were devoid of osmiophilic granules, no changes in the ultrastructure of subcellular organelles were seen. No cells were observed showing ultrastructural changes characteristic for irreversible injury, i.e., Jennings' granules in the mitochondria, discontinuities in the plasma membrane, ultracellular clarifications, and nuclear pyknosis. The calcium localization was similar to that in nonischemic tissue, and calcium overload in mitochondria was never observed.

**Discussion**

**Experimental Model for Stunned Myocardium**

Revascularization of ischemic myocardium by surgical or nonsurgical means has become an important therapeutic goal. The outcome of the reperfused myocardium will depend on the severity of the ischemic insult as well as on its duration. Brief episodes of intense myocardial ischemia of less than 25 minutes, induced by total coronary occlusion in the dog and
resulting in complete loss of mechanical function, are associated with postischemic LV dysfunction called "stunned myocardium." After full reperfusion, functional recovery over time will be the rule. With longer episodes of coronary occlusion, beyond 30 minutes and longer, subendocardial necrosis will ensue, resulting in permanent loss of some contractile units. Thus, after prolonged ischemia due to total coronary occlusion, postischemic dysfunction is related to the combined effect of permanent loss of function due to irreversible damage of some myocytes, on the one hand, and of temporary loss of function due to stunning of some other myocardial cells, on the other hand. In this case, only partial recovery of regional function is to be expected.

A large number of experimental models of myocardial stunning are flawed by the fact that the reperfused myocardium under study contains a mixture of permanently damaged and temporarily stunned myocardium. In particular, metabolic studies have been performed mostly in experimental models of prolonged myocardial ischemia (>30 minutes), resulting in mixed tissue injury, partly necrotic and partly stunned. In most other studies performed in anesthetized open-chest as well as closed-chest animals, the ultimate recovery of regional myocardial function was never documented, thus leaving the possibility of mixed tissue injury unchallenged.

From the above discussion, it is obvious that a definite diagnosis of stunning implies 1) documentation of complete recovery of contractile function after full reperfusion and 2) proof of absence of histological evidence for myocardial necrosis. Thus, in the strict sense, this diagnosis can only be made in retrospect. Therefore, we designed our experimental protocol to monitor recovery of regional function over time up to 2 weeks as well as to obtain pathological confirmation for the absence of irreversible injury. Nevertheless, a slight but significant release of creatine kinase enzymes in plasma was observed. This is similar to the data obtained in another experimental animal model for stunned myocardium, i.e., a single 15-minute coronary occlusion in the conscious baboon, in which increases in plasma creatine kinase as well as in the MB isoenzyme were observed in the absence of necrosis, suggesting that severe ischemia resulting in myocardial stunning may reversibly alter cell membrane permeability sufficiently as to lose cell constituents with molecular weights up to 80,000.

Another feature of the present model deserves some emphasis. Although loss of contractile function in the territory of a critical coronary artery stenosis is increasingly recognized in clinical cardiology, little experimental information is available concerning the mechanical and metabolic fate in the case of underperfused myocardium due to severe stenosis with some residual antegrade flow and the effect of subsequent reperfusion. A major finding of this study is that myocardial ischemia induced by a 1-hour critical stenosis, severe enough to decrease subendocardial flow by 83% and resulting in loss of systolic wall thickening, is followed after reperfusion by 1) a prolonged period of postischemic LV dysfunction and 2) full recovery of function without evidence of free wall myocardial necrosis by histopathologic and electron microscopic examination. This observation is an extension of earlier studies reported by Matsuzaki et al showing that a less severe reduction in regional flow maintained for an extended period of 5 hours, during which regional systolic short-

Table 4. 14C Clearance of Palmitic Acid at Different Time Intervals During Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>6 Hours (n=8)</th>
<th>12 Hours (n=3)</th>
<th>24 Hours (n=5)</th>
<th>1 Week (n=8)</th>
<th>2 Weeks (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral</td>
<td>0.14±0.01</td>
<td>0.14±0.02</td>
<td>0.12±0.02*</td>
<td>0.14±0.01</td>
<td>0.15±0.04</td>
</tr>
<tr>
<td>Posterior</td>
<td>0.11±0.02*</td>
<td>0.12±0.02*</td>
<td>0.13±0.02*</td>
<td>0.13±0.01</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>Septal</td>
<td>0.15±0.02</td>
<td>0.15±0.02</td>
<td>0.15±0.01</td>
<td>0.14±0.01</td>
<td>0.20±0.05</td>
</tr>
<tr>
<td>Anterior</td>
<td>0.16±0.02</td>
<td>0.16±0.01</td>
<td>0.14±0.01</td>
<td>0.15±0.01</td>
<td>0.18±0.07</td>
</tr>
<tr>
<td>Ratio† (anterior/posterior)</td>
<td>1.48±0.06</td>
<td>1.28±0.08</td>
<td>1.16±0.08</td>
<td>1.10±0.05</td>
<td>1.01±0.30</td>
</tr>
</tbody>
</table>

n, Number of dogs. Values are mean±SEM. Averages of three regions of interest per segment are given.

* p<0.02 vs. normal segments.
† No statistical evaluation because of different dogs in different groups.

Table 5. 14C Clearance of Acetate at Different Time Intervals During Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>6 Hours (n=11)</th>
<th>1 Week (n=4)</th>
<th>2 Weeks (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral</td>
<td>0.20±0.02</td>
<td>0.16±0.04</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td>Posterior</td>
<td>0.16±0.01*</td>
<td>0.16±0.03</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>Septal</td>
<td>0.21±0.02</td>
<td>0.15±0.04</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>Anterior</td>
<td>0.21±0.02</td>
<td>0.15±0.04</td>
<td>0.19±0.04</td>
</tr>
<tr>
<td>Ratio† (anterior/posterior)</td>
<td>1.36±0.12</td>
<td>1.07±0.10</td>
<td>1.00±0.31</td>
</tr>
</tbody>
</table>

n, Number of dogs. Values are mean±SEM. Averages of three regions of interest per segment are given.

* p<0.02 vs. normal segment.
† No statistical evaluation because of different dogs in different groups.
This model and the present observation may be relevant to the clinical situation of unstable angina, which is usually induced by a critical stenosis resulting in decreased but preserved resting antegrade flow.

**Metabolic Imaging**

The sequence of metabolic events on induction of ischemia has been extensively studied in animals as well as in vitro experimental systems, and the key role of anaerobic glycolysis has been recognized. The rate of lactate production and its removal from the cell determines how long and at what rate glycolysis can be maintained. The glucose is derived from endogenous sources (i.e., glycogenolysis) and from exogenous glucose uptake as the glycogen stores become eventually depleted.

The metabolic derangements associated with postischemic dysfunction have received far less attention, and a large number of studies were performed in ischemic models of mixed tissue injury. The present PET measurements of fatty acids and overall oxidation in true stunned myocardium show a significant decrease in metabolism relative to the remote normal zones. These abnormalities are prolonged despite full reperfusion and normal substrate availability.

**Overall oxidative metabolism.** The quantitative analysis of myocardial oxygen consumption using [1-14C]acetate has been extensively validated both in normal myocardium and ischemic myocardium as well as in reperfused myocardium. PET has offered new possibilities in this study, since AC has been validated as a quantitative tracer of myocardial tricarboxylic acid cycle flux and, hence, of oxygen consumption. The present animal model allowed for simultaneous analysis of regional mechanical function and regional oxygen consumption in the same segment by using dynamic measurements of AC kinetics with PET. These results confirm that the reduced contractile function in reperfused stunned segments is associated with a reduced oxygen consumption despite normalization of regional blood flow. This is in accordance with other studies.

These observations suggest that the normal relation between regional function and oxygen consumption has not changed and that the decrease in oxygen consumption in stunned myocardium is merely secondary to the decrease in mechanical work. These findings corroborate other studies using PET and also the observation by Schaper et al., who used regional venous blood sampling, a more direct measurement of oxygen consumption. However, other investigators have found the opposite result; i.e., myocardial oxygen consumption in stunned myocardium is unchanged or even increased. Two major differences in the protocol design of the latter studies are worth noting: 1) the early timing when measurements were made after reperfusion and 2) the severity in regional dysfunction observed, which was much different from our study. Indeed, in the latter studies, myocardial segments were still akinetic or even dyskinetic at least during the first third of systole, when oxygen consumption measurements were made. This brings up an interesting point. As early as 1932, Feng reported in isolated muscle that increasing stretch resulted in a proportional increase in heat production reflecting increased metabolism.
It is conceivable that the normal close coupling between mechanical work and oxygen consumption may be qualitatively different in mildly stunned segments with preserved systolic contraction as observed in our model compared with akinetic or dyskinetic segments in studies supporting an increased oxygen consumption. In support of this view, we offer from our laboratory the recent observation that moderately stunned myocardial segments displaying a decreased myocardial oxygen consumption were made to bulge by rapid asynchronous right ventricular pacing in the absence of regional ischemia and exhibited a disproportionate increase in oxygen consumption.59

Oxidation of fatty acids. A decreased slope of the early clearance phase of CPA kinetics was found during ischemia by several authors16,34,36,54 and shown to represent an impaired transfer of palmitoyl coenzyme A into mitochondria and greater “trapping” of CPA in the triglyceride pool.55 Back diffusion of nonmetabolized tracer may result in a paradoxical increase of the early clearance slope, leading to an underestimation of the decrease in fatty acid oxidation.35,36 Thus, the present data suggest that a decrease in the oxidation rate of exogenous fatty acids is also observed during postischemic dysfunction. Earlier PET studies combining CPA kinetics and glucose uptake using [2-18F]fluorodeoxyglucose have shown a marked enhanced regional uptake of the glucose analogue in the area of delayed CPA clearance and suggest that postischemic myocardium shifts substrate utilization from fatty acids to carbohydrates for its aerobic metabolism.17 It is important to note, however, that kinetic PET studies with CPA only assess relative differences in regional fatty acid metabolism and that, in the absence of an appropriate tracer kinetic model, blood and tissue time–activity curves cannot be translated into absolute substrate consumptions. The fact that in stunned myocardium the decrease in AC slope was proportionally greater than the decrease in CPA slope could be taken as indirect evidence that fatty acids still remain the substrate of choice of the reperfused myocardium, as shown by others.14,15 The quantitative contribution of fatty acid oxidation to the overall oxidative metabolism can only be obtained directly by steady-state infusion of 13C- and/or 14C-labeled substrates and arteriovenous measurements.19,56 These studies have shown sustained nonoxidative metabolism of glucose rather than a switch in substrate preference. Recently, Buxton and Schelbert57 have clearly demonstrated that the relative enhancement of glucose metabolism in postischemic myocardium was observed only when glucose metabolism in normal myocardium was low. The [2-18F]fluorodeoxyglucose signal in reversibly injured tissue is thus dependent on the glucose metabolic rate in the normal myocardium, which in turn is largely determined by the concentration of free fatty acids. This observation explains why reversibly injured tissue may or may not display the positive [2-18F]fluorodeoxyglucose signal in certain conditions.57

Time course of overall oxidative and fatty acid metabolism. Our experimental model and the protocol design offer a unique opportunity for longitudinal analysis of both regional myocardial function and regional overall oxidative metabolism as well as fatty acid oxidation in both normal and stunned zones. Repeated dynamic PET studies during a 2-week follow-up period showed a progressive normalization in overall oxidative metabolism and in fatty acid oxidation that paralleled the normalization of regional mechanical function as monitored by wall thickness measurements. At all times, regional flow as well as substrate availability remained normal.

Conclusions

Prolonged severe hypoperfusion of the myocardium during 1 hour of coronary artery stenosis elicits prolonged postischemic LV dysfunction. Simultaneous evaluation of changes in regional mechanical function as well as noninvasive metabolic imaging allow us to conclude the following: 1) Myocardial ischemia severe enough to produce loss of systolic contraction induced by 1-hour stenosis of a coronary artery, with minimal residual perfusion followed by full reperfusion, is associated with a prolonged period of postischemic dysfunction. Complete recovery of contractile function and the absence of cellular evidence for necrosis support the contention that the myocardium under study is stunned. 2) Decreases in regional mechanical function and regional oxygen consumption assessed with dynamic PET using 13C AC tracer are qualitatively similar in moderately stunned segments. 3) Stunned myocardial segments displayed a prolonged impairment in overall oxidative metabolism and in beta-oxidation of fatty acids, despite reoxygenation. This reduction in fatty acid metabolism was proportionally smaller than the decrease in overall oxidative metabolism, suggesting that fatty acids could still be the preferred substrate in normally perfused hypofunctioning segments. 4) Recovery of regional contractile function during a 2-week follow-up is paralleled by normalization in regional fatty acid metabolism and overall oxidative metabolism.

Acknowledgments

We thank Prof. I. Leusen for his continuous encouragement and helpful comments during completion of this study. We greatly appreciate the skillful technical assistance of W. Laneuva, D. Gyseleinck, C. Mabile, D. Ochrymovicz-Bemelmans, E. Michal, and R. Vanbutsele. We thank F. Cauw for analyzing the microsphere data and M. Cogneau, D. Labar, and their Cyclotron and Radiochemistry staff for providing the positron emitting tracers. We gratefully acknowledge the generous supply of etomidate (R 5100) from Janssen Research, Beerse, Belgium. Finally, the dedicated secretarial assistance of J. Cano is greatly appreciated.

References

6. Kuhn, W., H., Porterfield, J K, Weisman, H F, Weisfeldt, M L, Marban E: Pathophysiology and pathogenesis of stunned myocardium: Depressed Ca2+ activation of contraction as a consequence of...
What is the primary research focus of the document?


52. Feng TP: The effect of length on resting metabolism of muscle. J Physiol (Lond) 1932;74:441–454


Recovery of regional contractile function and oxidative metabolism in stunned myocardium induced by 1-hour circumflex coronary artery stenosis in chronically instrumented dogs.

G R Heyndrickx, W Wijns, D Vogelaers, Y Degrieck, A Bol, G Vandeplassche and J A Melin

Circ Res. 1993;72:901-913
doi: 10.1161/01.RES.72.4.901

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/72/4/901