Mechanisms of Chronic Regional Postischemic Dysfunction in Humans

New Insights From the Study of Noninfarcted Collateral-Dependent Myocardium

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Background. Even in the absence of a previous myocardial infarction, patients with coronary artery disease often present with chronic regional wall motion abnormalities that are reversible spontaneously or after coronary revascularization. In these patients, regional dysfunction has been proposed to result either from prolonged postischemic dysfunction (myocardial “stunning”) or from adaptation to chronic hypoperfusion (myocardial “hibernation”). This study examines which of these two mechanisms is responsible for the chronic regional dysfunction often detected in patients with angina and noninfarcted collateral-dependent myocardium.

Methods and Results. Twenty-six anginal patients (19 men; mean age, 60±9 years old) with chronic occlusion of a major coronary artery but without previous infarction were studied. Positron emission tomography was performed to measure absolute regional myocardial blood flow with $^{15}$N-ammonia at rest ($n=26$) and after intravenous dipyridamole ($n=11$). The kinetics of $^{18}$F-deoxyglucose and $^{13}$C-acetate were measured to calculate the rate of exogenous glucose uptake and the regional oxidative metabolism ($n=15$). Global and regional left ventricular function was evaluated by contrast ventriculography at baseline ($n=26$) and after revascularization ($n=12$). Transmural myocardial biopsies from the collateral-dependent area were obtained in seven patients during bypass surgery and analyzed by optical and electron microscopy. According to resting regional wall motion, patients were separated into groups with and without dysfunction of the collateral-dependent segments. In patients with normal wall motion ($n=9$), regional myocardial blood flow, oxidative metabolism, and glucose uptake were similar among collateral-dependent and remote segments. By contrast, in patients with regional dysfunction ($n=17$), collateral-dependent segments had lower myocardial blood flow ($77±25$ versus $95±27$ mL·min$^{-1}$·100 g$^{-1}$, $p<0.001$), smaller k values (slope of $^{13}$C clearance reflecting oxidative metabolism, $0.049±0.015$ versus $0.068±0.020$ min$^{-1}$, $p<0.001$) and higher glucose uptake (relative $^{18}$F-deoxyglucose-to-flow ratio of $1.9±1.6$ versus $1.2±0.2$, $p<0.05$) compared with remote segments. However, myocardial blood flow and k values were similar among collateral-dependent segments of patients with and without segmental dysfunction. After intravenous dipyridamole, collateral-dependent myocardial blood flow increased from $78±5$ to $238±54$ mL·min$^{-1}$·100 g$^{-1}$ in three patients with normal wall motion and from $88±17$ to only $112±44$ mL·min$^{-1}$·100 g$^{-1}$ in eight patients with regional dysfunction. There was a significant ($r=-0.85$, $p<0.001$) inverse correlation between wall motion abnormality and collateral flow reserve. Analysis of the tissue samples obtained at the time of bypass surgery showed profound structural changes in dysfunctioning collateral-dependent areas, including cellular swelling, loss of myofibrillar content, and accumulation of glycogen. Despite these alterations, the regional wall motion score improved significantly in the patients studied before and after revascularization (from $3.8±1.3$ to $0.8±0.9$, $p<0.005$).

Conclusions. In a subgroup of patients with noninfarcted collateral-dependent myocardium, immature or insufficiently developed collaterals do not provide adequate flow reserve. Despite nearly normal resting flow and oxygen consumption, these collateral-dependent segments exhibit chronically depressed wall motion and demonstrate marked ultrastructural alterations on morphological analysis. We propose that these alterations result from repeated episodes of ischemia as opposed to chronic hypoperfusion and represent the flow, metabolic, and morphological correlates of myocardial “hibernation.” (Circulation 1993;87:1513–1523)

Key Words • myocardial stunning • myocardial hibernation • myocardial metabolism • positron emission tomography • collateral-dependent myocardium • collateral flow reserve
Be cause of the necessary aerobic nature of normal cardiac metabolism, reductions in myocardial blood flow, or ischemia, invariably result in immediate loss of contraction. Therefore, the presence of chronic dysfunction was believed to represent irreversible damage due to myocardial necrosis followed by scar formation. The study of reperfusion of chronically dysfunctioning myocardium has revealed that contraction could resume to a given extent in some instances.1 This implies that during and after the ischemic episode(s), irreversible damage can be prevented by medium and/or long-term adaptive changes in myocardial perfusion and metabolism.2 These changes would lead to a different state of "perfusion-contraction matching,"3 among which "stunning"4,5 and "hibernation"6,7 have received much attention over the recent years.8

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In the present study, we attempted to elucidate which of these two mechanisms is responsible for the chronic regional dysfunction often detected in coronary patients despite the absence of previous myocardial infarction. We selected patients with angina pectoris and chronic occlusion of a major coronary artery. In this model, the myocardium normally supplied by the occluded vessel becomes totally dependent on the collateral flow. We used the quantitative capability of positron emission tomography to measure absolute myocardial blood flow,7,8 and to detect noninvasively the metabolic hallmarks of ischemic and postischemic states.9,10

Methods

Study Population

The study population consisted of 26 patients (19 men and seven women; mean age, 60±9 years; range, 46–75 years) with angina pectoris and well-defined coronary anatomy. By reviewing all diagnostic coronary angiograms performed at two clinical sites, we selected prospectively patients with complete occlusion of a proximal coronary artery retrogradely filled by collateral vessels. Twenty-five patients showed occlusion of the left anterior descending, among whom two had a totally occluded right and one had an occluded left circumflex coronary artery. The remaining patient had a proximal circumflex and right coronary artery occlusion with a normal left anterior descending artery. None of the patients had left main stenosis. Importantly, no patient had suffered from a previous myocardial infarction by clinical or enzymatic criteria, and standard 12-lead ECGs showed no abnormal Q waves.

Cardiac Catheterization

Selective coronary arteriography was performed from the femoral artery using preshaped catheters. Significant disease was defined as a >70% luminal diameter stenosis in any major coronary branch. The collateral circulation was graded as follows: 0, no visible collaterals; 1, poor (threadlike, poorly opacified distal arterial segment); 2, fair (good distal arterial segment lightly and slowly opacified); and 3, adequate (good distal arterial segment, normally and quickly opacified). Contrast left ventriculography in the 30° right anterior oblique projection was performed in every patient at baseline. Left ventricular volumes were measured from the first well-opacified sinus beat, excluding premature depolarization and the subsequent sinus beat. Left ventricular volumes were calculated at end diastole (R wave of the ECG) and end systole (minimal volume) using a standard Simpson's method. The regional ventricular wall motion was reviewed by two experienced observers. Each ventricular silhouette was divided into five segments (anterobasal, anterolateral, apical, inferior, and posterobasal). Regional wall motion in each segment was defined as hyperkinetic (−1), normal (0), hypokinetic (1), akinetic (2), or dyskinetic (3). A regional wall motion score for the collateral-dependent segments was calculated by summing the two anterior and apical segments in patients with left anterior descending coronary occlusion; the opposite segments plus the apex were summed in the patient with circumflex and right coronary occlusion. In the patients undergoing revascularization, by either bypass surgery (n=11) or percutaneous transluminal coronary angioplasty (n=12), a functional follow-up was requested. Coronary angiography and contrast left ventriculography 5–8 months after revascularization were obtained in 12 patients with chronic dysfunction. The same analysis of global and regional ventricular function was performed. In seven patients with left anterior descending coronary artery occlusion who were undergoing bypass surgery, a transmural needle biopsy specimen was obtained from the anterior myocardium and analyzed for morphological changes. This study protocol was approved by the ethical committee of our institutions, and no complications resulted from any part of the study.

Positron Emission Tomography

Positron emission tomography was performed an average of 14 days (range, 1–48 days) after cardiac catheterization. Acquisitions were performed with an ECAT III (CTI, Knoxville, Tenn.) one-ring tomograph, the characteristics of which have been described previously.11 Measurements were performed with a stationary ring, and images were reconstructed with an in-plane resolution of 8-mm full-width-at-half-maximum (FWHM). The collimator aperture was set at 30 mm, resulting in a slice thickness of 15 mm FWHM. Regular calibration of the tomograph versus a well counter was performed by measuring a uniform cylindrical phantom (diameter, 20 cm) filled with a solution of 68Ge. All patients were studied after overnight fasting. To standardize the dietary state and enhance the myocardial glucose uptake, a venous line was inserted into the antecubital vein, and the patient was continuously perfused with 10% dextrose-in-water solution (15 μmol·kg⁻¹·min⁻¹). Each patient was carefully positioned in the tomograph. Serial transmission scans at different levels were obtained to allow subsequent correc-
tion for photon attenuation. All transmission scans were viewed before collection of emission data to verify proper positioning of the patient. Correct positioning was maintained throughout the study with the use of a light beam and indelible felt pen marks on the patient torso.

Myocardial perfusion was assessed with $^{13}$N-ammonia, myocardial oxidative metabolism with $^{1}$C-acetate, and exogenous glucose uptake with $^{18}$F-deoxyglucose (FDG). Fifteen patients had flow and metabolic imaging at rest using the three tracers. In the remaining 11 patients, myocardial blood flow was studied at baseline and after intravenous administration of dipyridamole (0.56 mg/kg over 4 minutes, followed by 0.28 mg/kg over 6 minutes) to assess myocardial flow reserve in remote and collateralized segments. Heart rate, arterial blood pressure, and 12-lead surface ECG were monitored during dipyridamole infusion. All tracers were injected intravenously by means of an infusion pump (model 351, Sage Instruments). After collection of attenuation data, 10–15 mCi $^{13}$N-ammonia was injected over a 20-second period. Beginning with tracer injection, 28 serial cross-sectional images were acquired in a decay-compensated mode for 10 minutes. After a 50-minute interval for decay of $^{13}$N radioactivity to baseline levels, 10–15 mCi $^{1}$C-acetate was injected over a 20-second period. Beginning with tracer injection, 25 serial cross-sectional images were acquired for 25 minutes. After an additional 60-minute interval (three times the $^{1}$C period) for decay of $^{1}$C radioactivity to baseline, 15 mCi $^{18}$F-FDG was infused over 60 seconds, followed by acquisition of 34 serial cross-sectional images for 45 minutes.

For analysis, each reconstructed tomographic image was corrected for physical decay. Nine 3.6-cm$^2$ volumes of interest were assigned to each image of the left ventricular myocardium, and another volume was assigned to the center of the left ventricular blood pool. Three of these volumes of interest were located in the interventricular septum, three others in the anterior wall, and the remaining three in the lateral free wall of the left ventricle. For the measurement of hyperemic blood flow, three large regions, each representing the average of the three smaller regions, were used. Counts were corrected for partial volume and spillover effects using a specially developed Monte-Carlo simulation, as well as for dead time losses.

Circumferential profiles. $^{13}$N-Ammonia and $^{18}$F-FDG cross-sectional images were analyzed with an operator-interactive computer program using circumferential profiles. The program normalized $^{18}$F and $^{13}$N counts within a given myocardial cross section to maximal activity in the same ventricular slice. Each cross section of the left ventricle was then divided into serial 30$^\circ$ segments. Activity within each segment was expressed as percent of maximal activity and subsequently normalized to peak $^{13}$N-ammonia segmental activity at the same level. In segments with $<$80% of maximal ammonia activity in the same slice, a pattern of flow-metabolism "mismatch" was considered to be present when the relative segmental FDG/ammonia activity ratio exceeded 1.2.13 Regions of interest in tomograms obtained after administration of $^{13}$N-ammonia were subdivided to encompass regions of interest in remote and collateralized tissue. Remote (normal) regions were usually identified as the four 30$^\circ$ segments from the lateral free wall and the basal septum in patients with occlusion of the left anterior descending coronary artery and patent right and circumflex arteries. Collateralized regions were identified as the three 30$^\circ$ segments from the anterior wall in patients with left anterior descending coronary artery occlusion and the three 30$^\circ$ segments from the lateral free wall in the two patients with circumflex coronary artery occlusion.

The quantitation of tomographic data was performed as previously described in our laboratory. One transaxial slice per patient was analyzed for dynamic studies. The regional myocardial perfusion was quantified by use of a three-compartmental model developed by Hutchins et al.7 No correction for circulating metabolites was applied. The kinetics of $^{1}$C-acetate were used to evaluate tricarboxylic acid cycle turnover.14–15 After tracer injection, $^{1}$C radioactivity clears exponentially from the myocardium, and the first exponential clearance rate reflects immediate oxidation of acetate. Multiexponential least-squares routines were used to fit the regional time-activity curves and to calculate the clearance slopes and half-times of the curve components. As in previous human studies, $^{13}$C clearance from the myocardium under resting conditions was always monoexponential. Finally, a three-compartmental FDG tracer kinetic model and the Patlak graphic analysis were used to estimate regional myocardial glucose uptake.16

Venous blood samples for determination of plasma glucose, lactate, fatty acids, and insulin were obtained at the end of the $^{18}$F-FDG study in the 15 patients undergoing metabolic imaging.

Morphological Tissue Analysis
A transmural needle biopsy (20-mm 14-gauge Trucut, Travenol) was obtained at the time of surgery, when the heart had been exposed, and before cardiopulmonary bypass. The site of the biopsy was selected in the operating theatre (by either J.A.M. or W.W.) in the anterolateral free wall between the left anterior descending and the first or second diagonal artery depending on the level showing metabolic changes on positron emission tomography. Immediately after the biopsy was performed, the specimens were separated into subendocardial and subepicardial parts and immersed in 3% glutaraldehyde fixative, buffered to pH 7.4 with 0.1 M sodium dihydrogenophosphate. The tissue fragments were postfixed for 1 hour at 4°C with 1% osmium tetroxide, buffered to pH 7.4 with 0.05 M veronal acetate containing 93 mM sucrose, subsequently dehydrated in ethanol, and embedded in Epon (for electron microscopy). Light microscopy was used to screen for interesting zones to be observed by electron microscopy and, second, as a morphometric method to quantify cellular alterations and the percent connective tissue. Semithick toluidine blue-stained sections were obtained from both subendocardial and subepicardial parts of the biopsy. Each section was examined using a special grid with vertical and horizontal lines dividing the field into 121 identical squares. Considering the entire grid to represent 100%, it was possible to measure the percent connective tissue by the number of squares surrounding dense connective tissue. A value of more than 5% connective tissue was considered abnormal because this cannot be accounted for by the connective tissue normally interspersing myocardial cells and surrounding blood vessels. This procedure was repeated several times on different zones of the biopsy.
Subsequently, 100–200 cardiomyocytes sectioned through their nuclei were examined and separated into two categories: cardiomyocytes appearing normal and cardiomyocytes presenting loss of myofilbrils and accumulation of cytoplasmic dark-blue material.\textsuperscript{17} Periodic acid-Schiff staining then was performed to confirm that the dark-blue material seen with toluidine blue staining is glycogen. Thus, by light microscopy, it was possible to quantify the percent connective tissue, the percent surface occupied by cells, and, within this surface, the percent cardiomyocytes with and without structural alterations. Thin sections were stained with uranyl acetate and lead citrate before examination with electron microscopy (Philips EM300). This method was used to qualify the structural alterations already suspected with light microscopy. As previously reported,\textsuperscript{17} the ultrastructural features of these modified myocytes are loss of myofilbrillar content, accumulation of glycogen, cellular swelling, irregular nuclear shape, and abnormalities in the size and shape of the mitochondria. The nucleus usually appears active, and the plasma membrane is normal.

Statistical Analysis

Values are expressed as mean±1 SD, unless otherwise stated. For statistical analysis, least-squares regression routines, the Spearman rank correlation test, and the Student’s t test for paired or unpaired data were used. ANOVA was used to compare the multiple regional data. Values of p<0.05 were considered statistically significant.

Results

Clinical and Angiographic Patient Characteristics

According to resting regional wall motion in collateral-dependent segments (Table 1), the patients were divided into two groups: Group 1 consisted of nine patients (eight men; mean age, 60±7 years; range, 49–73) with normal or minimal regional wall motion abnormalities (mean regional wall motion score, 0.4±0.7; range, 0–2), and group 2 consisted of 17 patients (11 men; mean age, 61±9 years; range, 46–75) with severe regional dysfunction (mean regional wall motion score, 4.5±1.2; range, >2–7). Figure 1 shows the wall motion score along the ventricular silhouette in the patients with left anterior descending coronary occlusion from both groups. Angiographic collateral grade was similar among the two groups. There was a nonsignificant (p=0.12) trend toward more severe anginal symptoms (Canadian Heart Association class III or IV) in patients with dysfunctioning collateral-dependent myocardium.

Baseline Regional Myocardial Blood Flow and Metabolism

The baseline absolute myocardial blood flow was measured in all 26 patients and is shown in Figure 2 in both remote and collateral-dependent myocardial regions.

No regional differences were found in patients with normal resting regional wall motion. In patients with abnormal resting wall motion, myocardial blood flow was higher in remote than collateral-dependent segments (95±27 versus 77±25 mL·min\textsuperscript{-1}·100 g\textsuperscript{-1}, p<0.001). However, no difference was found among collateral-dependent segments from patients with and without abnormal wall motion (77±25 versus 85±14 mL·min\textsuperscript{-1}·100 g\textsuperscript{-1}, p=NS). Figure 3 shows the same data but compares the flow profile along the tomographic image in the patients with left anterior descending coronary occlusion. The only significant (p<0.01) difference relates to the increased flow in the septal and lateral walls relative to the anterior wall in the patients with dysfunctioning collateral-dependent myocardium.

Table 2 compares myocardial blood flow, absolute glucose uptake, relative 18F-FDG to 13N-ammonia circumferential profiles, oxygen consumption, and mean

\begin{table}
\centering
\caption{Clinical and Angiographic Data}
\begin{tabular}{|c|c|c|}
\hline
 & Group 1 & Group 2 \\
\hline
Wall motion in collateral-dependent segments & Normal & Abnormal \\
\hline
No. of patients & 9 & 17 \\
Age (years) & 60±7 & 61±9 \\
Sex & 8M, 1F & 11M, 6F \\
Anginal class & & \\
CHA I+II & 8/9 & 10/17 \\
CHA III+IV & 1/9 & 7/17 \\
Diseased vessels & & \\
LAD & 8/9 & 13/17 \\
LAD+Cx & - & - \\
LAD+RCA & 1/9 & 2/17 \\
LAD+RCA+Cx & - & 1/17 \\
Cx+RCA & - & 1/17 \\
Collateral score & 2.3±0.5 & 2.2±0.6 \\
Rate-pressure product (mm Hg/bpm) & 8,871±2,637 & 9,368±1,035 \\
LV end-diastolic index (mL/m\textsuperscript{2}) & 92±22 & 112±17 \\
LV end-systolic index (mL/m\textsuperscript{2}) & 27±10 & 47±13 \\
LV ejection fraction (%) & 68±9 & 52±13 \\
Regional wall motion score & 0.4±0.7 & 4.5±1.2 \\
\hline
\end{tabular}
\end{table}

CHAS, Canadian Heart Association; Cx, circumflex artery; LAD, left anterior descending coronary artery; LV, left ventricle; RCA, right coronary artery; bpm, beats per minute.

No statistical analysis was applied to the differences in left ventricular function, which are built in by study design.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Plot of mean wall motion score along the ventricular silhouette is shown for patients from group 1 (●) and group 2 (■). There is no significant difference in the regional wall motion score in group 1. No statistical analysis was applied to the differences between groups, which is built in by study design.}
\end{figure}
estimated wall thickness (by use of the Monte Carlo simulation) in a subgroup of 15 patients. Absolute myocardial flow values in this subgroup of patients were similar to those measured in the total population. The metabolic rates for exogenous glucose utilization in remote and collateral-dependent myocardium were highest in dysfunctioning patients, 42±20 and 38±16 μmol·min⁻¹·100 g⁻¹, respectively. Identical values were found between remote and collateralized regions in group 2 patients (33±12 μmol·min⁻¹·100 g⁻¹). The ratio of metabolic rate for glucose utilization to myocardial blood flow, reflecting the extraction of glucose by the myocardium, was also higher in dysfunctioning collateral-dependent compared with normally contracting collateral-dependent segments (0.60±0.27 versus 0.42±0.16 μmol/mL, p<0.004). The relative ¹⁵N-ammonia uptake in collateralized segments averaged 83±10% (range, 60–98% of maximum) in group 1 and 65±17% (range, 21–93%) in group 2 (p<0.001). The ¹⁸F-FDG uptake in collateral-dependent segments relative to the flow profile was significantly higher in patients with compared to patients without wall motion abnormalities ("mismatch" ratio, 1.9±1.6 versus 1.2±0.2, p<0.05). A typical example of these findings is illustrated in Figure 4 in a patient with akinesia of the anteroapical wall.

The ¹³C-acetate kinetics in remote and collateral-dependent myocardium were analyzed by use of multiexponential least-squares fitting routines. The ¹³C clearance was monoeponential in both remote and collateralized segments. In patients with normal resting wall motion, the monoeponential slope of ¹³C-acetate clearance, k, was comparable between remote and collateralized segments. At variance, in patients with resting wall motion abnormalities, ¹³C-acetate clearance was faster in remote than in collateral-dependent segments (0.068±0.020 versus 0.049±0.015 min⁻¹, p<0.001). Acetate kinetics did not differ, however, significantly among collateral-dependent segments of patients with and without regional wall motion abnormalities. The venous plasma glucose, fatty acids, lactate, and insulin plasma concentrations at the time of the tomographic study are listed in Table 3.

**Collateral-Dependent Myocardial Flow Reserve**

The absolute myocardial blood flow during dipyridamole infusion was determined in a subgroup of 11 patients with total occlusion of the left anterior descending coronary artery and normal circumflex coronary artery. Infusion of dipyridamole induced anginal pain and significant ST segment depression (>0.1 mV in lateral leads) in all patients. In three patients with normal baseline anterior wall motion, transmural myocardial blood flow in collateral-dependent segments increased from 78±5 to 238±54 mL·min⁻¹·100 g⁻¹ during hyperemia. At variance, in eight patients with dysfunctioning collateral-dependent myocardium, the flow increase was severely blunted from 88±17 at rest to 112±44 during hyperemia, as illustrated on Figure 4. The maximal flow was not different in remote segments between group 1 (274±44) and group 2 (279±125) patients. The "collateral flow reserve," defined as the ratio of hyperemic to basal flow, is shown in Figure 5. A significant inverse relation (r=-0.85, p<0.001) was found between the baseline anterior wall motion score and the ratio of hyperemic to basal flow in collateral-dependent segments (Figure 6). There was, however, no relation between the baseline wall motion score and the resting myocardial blood flow (r=-0.19) in these segments (Figure 6).

**Functional Follow-up**

Coronary angiography and contrast left ventriculography 5–8 months after coronary revascularization were available in 12 patients from group 2. Among these, eight patients underwent percutaneous transluminal coronary angioplasty of the occluded vessel, and four had coronary artery bypass surgery. Adequate revascularization of the dysfunctioning segments was achieved in 11 patients. In the remaining, reoclusion of the left anterior descending coronary artery had occurred after an initially successful angioplasty. Nevertheless, the regional wall motion score improved in all patients, including the patient with reoclusion of the left anterior descending coronary artery (from 3.8±1.3 to 0.8±0.9, p<0.005). The global ejection fraction improved (from 55±7% to 65±8%, p<0.001) and the end-systolic volume decreased (from 47±10 to 34±11 mL/m², p<0.005) in all patients.
TABLE 2. Myocardial Blood Flow, Exogenous Glucose Uptake, Oxidative Metabolism, and Mean Wall Thickness in Remote and Collateral-Dependent Myocardium

<table>
<thead>
<tr>
<th>Group 1 (n=6 patients)</th>
<th>MBF</th>
<th>rMGU</th>
<th>FDG/NH₃</th>
<th>k</th>
<th>Wall thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remote segments (n=25)</td>
<td>78±8*</td>
<td>33±12*</td>
<td>1.1±0.1</td>
<td>0.058±0.008*</td>
<td>13.3±0.8</td>
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<tr>
<td>Collateralized segments (n=18)</td>
<td>75±16†</td>
<td>33±11*</td>
<td>1.2±0.2</td>
<td>0.054±0.010*</td>
<td>13.1±0.6</td>
</tr>
<tr>
<td>Group 2 (n=9 patients)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Remote segments (n=35)</td>
<td>92±23</td>
<td>42±20</td>
<td>1.3±0.6</td>
<td>0.068±0.020†</td>
<td>13.4±0.5</td>
</tr>
<tr>
<td>Collateralized segments (n=30)</td>
<td>68±17†</td>
<td>38±16</td>
<td>1.9±1.6*</td>
<td>0.049±0.015†</td>
<td>12.1±1.1†</td>
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</tbody>
</table>

FDG/NH₃, ratio of ¹⁸F-deoxyglucose to ¹⁵N-ammonia relative uptake; k (min⁻¹), slope of ¹⁴C-acetate clearance; MBF (mL · min⁻¹ · 100 g⁻¹), myocardial blood flow; rMGU (µmol · min⁻¹ · 100 g⁻¹), regional myocardial glucose uptake.
*p<0.05, †p<0.01, ‡p<0.001 vs. remote segments of group 2.

Structural Abnormalities in Collateral-Dependent Myocardium

Macroscopic scars were not detected during surgery in the anterior wall. The morphological changes observed in a patient with anterior wall dysfunction are illustrated in Figure 7. The altered cells are characterized by a severe reduction of contractile filaments, accumulation of glycogen, reduction of sarcoplasmic...
reticulum, and appearance of numerous small mitochondria. The ultrastructure of the above-mentioned subcellular organelles is normal. The group data are shown in Table 4. The patients are ranked according to decreasing severity of anterior wall motion abnormalities. With the exception of patient A, there is little fibrosis, and a large proportion of myocardial cells show ultrastructural changes as illustrated, particularly in patients with chronic regional dysfunction.

**Discussion**

In the present study, we attempted to elucidate the mechanisms by which ischemia may result in chronic regional dysfunction of viable noninfarcted myocardium. We selected a human model in which myocardial perfusion depended entirely on collateral blood supply and in which regional dysfunction met the clinical criteria for chronic hibernation, as defined by Rahimtoola.1 The findings can be summarized as follows. 1) Dysfunctioning collateral-dependent segments show reduced myocardial blood flow and acetate clearance while exogenous glucose uptake is maintained compared with remote normally perfused segments in the same patient. 2) There is no significant difference between normally contracting and dysfunctioning collateral-dependent areas with respect to baseline blood flow and oxygen consumption. 3) Immature and/or insufficiently developed collaterals do not provide adequate flow reserve in the subgroup of patients with chronic regional dysfunction. 4) Marked morphological alterations, including a substantial loss of myofibrillar content, represent the structural correlate of the chronic dysfunction and of the metabolic changes.

Despite a marked reduction in external work, dysfunctioning collateral-dependent myocardium remains metabolically active as demonstrated by the nearly normal baseline flow and oxygen consumption and by the increased 18F-FDG uptake relative to flow. Thus, the data in this model do not support the hypothesis that chronic dysfunction results from a chronic reduction in resting flow. The contention that flow is decreased in “hibernating” states has been based on clinical studies of the relative distribution of flow tracers such as 201TI or 82Rb.18 The interpretation of these scintigrams usually assumes that the segments with maximum tracer uptake have normal flow. However, perfusion scintigraphy provides only estimates of relative differences in tracer distribution. A seemingly decreased perfusion to a dysfunctional segment, as seen on Figure 4, may result from an absolute increase in flow to the remote hyperfunctioning tissue, as shown in the present study by the quantitative measurements of transmural blood flow with 13N-ammonia.

Although a significant reduction in resting flow can be ruled out, some metabolic changes are nevertheless apparent in the patients from group 2. The glucose uptake is significantly increased in the remote areas, consistent with an increase in regional workload,19 although slight differences in substrate supply also might have contributed. In the dysfunctioning collateral-dependent areas, the absolute glucose uptake is preserved and the relative glucose uptake is significantly increased, a pattern called flow-metabolism “mismatch.”20 This pattern has been shown to identify reversible tissue injury after transient ischemic insults.18,20,21 After adequate revascularization, improvement of regional wall motion is expected to occur in 78–92% of such areas, which indicates persistent tissue viability.18,20,21

The flow reserve of collateral-dependent myocardium was evaluated using diprydamole as the hyperemic agent.22 A wide range of hyperemic transmural flow values, from a fourfold increase to a 20% decrease in basal flow, was found. This is consistent with animal studies showing heterogeneity of the collateral vascular system early after total coronary occlusion induced by placement of an amiodar constrictor on a proximal coronary artery.23–25 Whether the blunted collateral reserve in patients with regional dysfunction represents immaturity or insufficiency of the collateral system cannot be assessed from the present clinical data because the exact time of the coronary occlusion, the delay between the occlusion and the study, and the individual rate of collateral growth are unknown. The lack of correlation between the angiographic collateral score and the absolute tissue perfusion values is not surprising and probably relates to the limited resolution of angiography. Another explanation could be the presence of functional extracoronary collaterals in these patients.26 Although all patients had angina pectoris, the anginal symptoms tended to be more severe in patients with chronic dysfunction and reduced collateral flow reserve.

**Table 3. Plasma Substrate Levels**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>Glucose (mM)</td>
<td>6.2±2.2</td>
<td>7.2±2.4</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>36±14</td>
<td>35±14</td>
</tr>
<tr>
<td>Fatty acids (µM)</td>
<td>793±108</td>
<td>713±359</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>0.95±0.24</td>
<td>0.83±0.19*</td>
</tr>
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</table>

*p<0.05 group 1 vs. group 2.

**Figure 5. Bar graph of ratio of hyperemic to baseline absolute flow shown as an index of “collateral flow reserve,” which is significantly reduced in collateral-dependent (COLL.-DEP.) segments of group 2 patients. *p<0.001.**
Severe ischemia is likely to occur repeatedly in those patients during daily life caused by increased demand, reductions in blood pressure, spontaneous increases in microvascular constrictor tone, or a combination of any of these mechanisms. In patients with a significant increase in collateral-dependent flow, angina and objective signs of ischemia during dipyridamole infusion still occurred. Similar findings were reported in a recent clinical study in which collateral flow reserve was assessed in patients with total occlusion of the left anterior descending coronary artery by use of thermodilution of the great cardiac vein. In this study, collateral flow increased significantly in all patients during infusion of dipyridamole, and collateral flow reserve was within normal limits. As shown by Schaper and others in animal studies, underperfusion in that case usually is limited to the subendocardium because pharmacological vasodilation or moderate exercise can induce a transmural coronary steal.

The present findings challenge the currently accepted understanding of the pathophysiology of chronic dysfunction of noninfarcted myocardium. Several studies have documented the beneficial effects of coronary revascularization on survival and cardiac function in patients with coronary artery disease and left ventricular dysfunction. These observations have led to the speculation that chronically hypoperfused myocardium, called "hibernating," could maintain viability simply by reducing its metabolic demand to match the decreased supply for as long as myocardial perfusion was inadequate. The chronic impairment of contractile function in this setting was considered to represent a protective mechanism, minimizing the energy requirements and preventing the appearance of irreversible tissue damage. This concept requires achievement of a new steady state between reduced supply and decreased demand and implies the existence of chronic low-flow myocardial ischemia. Little experimental evidence supports the contention that myocardial ischemia can be maintained for a prolonged period of time while avoiding development of irreversible tissue injury. In conscious dogs undergoing partial occlusion of the circumflex coronary artery for 5 hours, Matsuzaki et al were able to achieve sustained reduction of systolic wall thickening, with only minimal necrosis limited to the papillary muscle. Bolukoglu et al recently achieved sustained reduction in segmental shortening without necrosis in swine undergoing a 50% reduction in left anterior descending coronary artery flow for 7 days. In anesthetized open-chest swine, Fedele et al showed that the metabolic hallmarks of ischemia tended to resolve over time despite persistent segmental dysfunc-
tion. Although these findings suggest that a precarious steady state between reduced oxygen supply and decreased oxygen demand can be achieved and maintained for some time under particular experimental conditions, no data are presently available to indicate that such a perfusion-contraction matching can persist for weeks or months in chronic animal preparations, let alone in the intact individual. Conversely, an overwhelming number of experimental data demonstrate that episodes of acute ischemia, followed by reperfusion, result in prolonged posts ischemic myocardial dysfunction, which recovers progressively over hours or days on adequate restoration of flow.3-6 The evidence that “myocardial stunning” does occur in patients with coronary artery disease was recently reviewed by Bolli4 and pertains to different clinical situations, such as unstable angina,35,36 reperfused infarction,37 or after cardiac surgery.38 Recovery from myocardial stunning may, however, be hampered by incomplete reperfusion, renewed episodes of myocardial ischemia, or both. In experimental animals, repeated episodes of alternating ischemia and reperfusion have been shown to induce prolonged myocardial dysfunction.39 One might thus hypothesize that rather than resulting from a state of decreased flow, chronic dysfunction could be due to episodes of severe ischemia, with the functional recovery incomplete because of renewed episodes. In this study, dysfunctioning collateral-dependent segments showed nearly normal baseline blood flow and oxygen consumption but severely limited flow reserve, which strongly supports such a scenario.

Finally, a definite answer was obtained from the analysis of tissue samples. Because experimental stunning is not associated with significant alterations in myocardial ultrastructure,5 we performed a detailed morphological analysis of dysfunctioning collateral-dependent human myocardium obtained during surgery in a limited number of patients. Profound abnormalities were found, very similar to those described as early as 1981 by Flameng et al.17 The most striking features observed in chronically hypokinetic segments were the loss in myofibrillar content and the excessive accumulation of glycogen. We believe that this peculiar morphological pattern represents the ultrastructural correlate of myocardial “hibernation.” Whether these structural changes are due to ischemia per se or represent an adaptation to chronic akinesia is not known. Interestingly, unloading of the cat papillary muscle by cutting its chordae was shown to induce myocyte dedifferentiation, which resembles in some aspects the observed pattern.40 Although this remains entirely speculative, it is likely that these structural changes are reversible after successful revascularization, as can be inferred from the recovery of wall motion over time. The comparison of the morphological data with the findings on metabolic imaging raises intriguing questions regarding the significance of increased glucose uptake in the presence of glycogen accumulation as well as on the mechanisms by which oxygen consumption is preserved, not only in the absence of contraction but almost in the absence of contractile apparatus.

Study Limitations

This study has several potential limitations that should be acknowledged. The study population was carefully selected to avoid inclusion of patients with previous infarction in the collateral-dependent areas. It cannot be ruled out that small foci of subendocardial necrosis were present in some instances. Although histologic data are available in a limited number of patients, the fact that significant fibrosis was seen only in one of seven cases is reassuring. No significant relation was found between the presence of wall motion abnormalities and the severity of angina from carefully taken history. It would have been of interest to compare the total ischemic burden in both patient groups because painless episodes may have contributed to the repeated stunning. It was not possible, however, to obtain Holter tape monitoring for quantitation of ST-T segment abnormalities. The quantitation capability of positron emission tomography is limited by its inability to accurately measure true tissue tracer concentrations when the thickness of the imaged object is less than twice the spatial resolution of the imaging device.41 Although thin-walled infarcted myocardium was not encountered in this study, the mean thickness of akinetic segments during the cardiac cycle still should be smaller than the thickness of remote normally contracting areas, leading to an underestimation of tracer concentration in the former segments. To circumvent this limitation, we recently developed a geometric approach that allows regional recovery coefficients to be computed.12,13 In addition, dynamic data are less sensitive to this measurement error. In any case, any underestimation of the true values in the dysfunctioning collateral-dependent segments would actually reinforce

| Table 4. Relation Among Anterior Wall Motion, Flow, Glucose Metabolism, and Quantitative Morphological Data |
|-------------------------------------------------|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Patient | AWMS | MBF (mL·min⁻¹·100 g⁻¹), myocardial blood flow | rMGU | FDG/NH₃, ratio of ¹⁸F-deoxyglucose to ¹³N-ammonia relative uptake | SE | TM | % Fibrosis | % AC |
| A* | 4 | 96 | 37 | 3.45 | 50 | – | 90 | – |
| B | 3 | 57 | 30 | 1.03 | 1 | 1 | 40 | 35 |
| C | 3 | 110 | 40 | 1.34 | 11 | 8 | 67 | 59 |
| D | 3 | 72 | 42 | 1.50 | 5 | 4 | 27 | 19 |
| E | 1 | 74 | 38 | 1.33 | 10 | 6 | 39 | 30 |
| F | 1 | 78 | 12 | 1.69 | 2 | 2 | 15 | 15 |
| G | 1 | 89 | 26 | 1.00 | 3 | 3 | 5 | 4 |

*Subepicardial sample not available in this patient.

AWMS, anterior wall motion score; MBF (mL·min⁻¹·100 g⁻¹), myocardial blood flow; rMGU (μmol·min⁻¹·100 g⁻¹), regional myocardial glucose uptake; FDG/NH₃, ratio of ¹⁸F-deoxyglucose to ¹³N-ammonia relative uptake; SE, subendocardial sample; TM, transmural data; % AC, percent abnormal cells.
our findings. Also due to the finite resolution of the equipment, only transmural data can be obtained, whereas measurements limited to the subendocardium would have been of great interest. A key finding in this study relates to the baseline blood flow measurements. For this purpose, we used 15O-Na-ammonia. The retention of this tracer is known to depend on metabolic trapping, which may be modified in postischemic tissue. It could be useful to confirm our findings using another tracer, such as 18O-labeled water. In animal studies, however, we found excellent correlations between both tracers and microsphere flow estimates. The clearance kinetics of 12C-acetate reflect the turnover of the tricarboxylic acid cycle, which provides an indirect estimate of oxygen consumption. Uncoupling between the tricarboxylic acid cycle and the respiratory chain cannot be ruled out. Therefore, further studies directly measuring the oxygen consumption using 15O-labeled oxygen are warranted.

The interpretation of the relation between morphological changes seen by microscopy and the metabolic abnormalities seen by imaging requires much caution. Although special care was taken to obtain representative tissue samples from the center of the ischemic area at the appropriate level, we realize that a 15-mg biopsy probably is 5,000 times smaller than the entire anterior ischemic area. Obviously, such a small sample size is imposed by safety considerations. Finally, the time course of functional recovery was not assessed. This would have been of great interest because myocardial hibernation was reported to improve immediately on revascularization. Given the severity of the observed ultrastructural changes, it would appear to be a very unlikely event, but this particular issue was not addressed.

Our findings have important clinical implications. First, we have shown that even absolute regional myocardial blood flow measurements, let alone relative flow distribution scintigrams, cannot differentiate among stunned or hibernating dysfunctioning segments. Overinterpretation of scintigraphic data has actually led to erroneous hypotheses regarding the pathophysiology of hibernation. To improve the clinical decision-making process in patients with poor left ventricular function, the ability to detect the presence of structural changes of the myocardium would represent a major advance. This goal challenges the less-than-ideal specificity of the currently available imaging techniques. Second, there is an ongoing controversy regarding the deleterious impact of reversible ischemia on prognosis, which is mainly determined by the ventricular function. As the present data show that renewed episodes of severe ischemia may result in severe chronic regional myocardial dysfunction, it is likely that the prognosis of these patients will be significantly improved by the reversal of ischemia after adequate coronary revascularization.

In conclusion, we have described a subgroup of patients with noninfarcted collateral-dependent myocardium in whom chronic regional dysfunction meets the clinical criteria for chronic hibernation (chronic dysfunction with hypoperfusion by relative perfusion imaging). In these patients, however, absolute myocardial blood flow and oxygen consumption in the dysfunctioning collateral-dependent segments are nearly normal at rest, collateral flow reserve is markedly blunted, and major structural changes are present on morphological analysis. In these patients, impaired function probably results from repetitive episodes of ischemia with a persistent stunning effect, which, at variance with experimental stunning, eventually results in ultrastructural alterations. We propose that these alterations represent the flow, metabolic, and morphological correlates of chronic myocardial hibernation.

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