Energy Stores and Metabolites in Chronic Reversibly and Irreversibly Dysfunctional Myocardium in Humans

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OBJECTIVES Our goal was to study metabolic energy stores and lactate content in chronic reversibly and irreversibly dysfunctional myocardium.

BACKGROUND It is unknown whether metabolism is deranged in chronic reversibly and irreversibly dysfunctional myocardium in humans. Semi-quantitative histological examinations have shown altered mitochondrial morphology and glycogen accumulation in dysfunctional regions.

METHODS We studied 25 patients with a mean ejection fraction of 38 ± 9% scheduled for coronary artery bypass surgery. Regional perfusion and metabolism were assessed by positron emission tomography, and regional function was assessed by echocardiography. Perioperative myocardial biopsies were obtained from a control region and from a dysfunctional region. We analyzed biopsies for contents of non-collagen protein (NCP), ATP, ADP, AMP, glycogen and lactate. Six months after surgery we assessed wall motion by echocardiography to group patients in those with (n = 11) and without (n = 14) functional improvement.

RESULTS Reversibly dysfunctional myocardium had reduced perfusion (0.59 ± 0.16 vs. 0.69 ± 0.20 ml/g/min, p < 0.05), similar glucose-tracer uptake (92 ± 12 and 95 ± 149%, ATP/ADP ratio (2.4 ± 1.1 and 2.4 ± 0.7), glycogen content (631 ± 174 and 362 ± 148 nmol/µg NCP) and lactate levels (59 ± 27 and 52 ± 29 nmol/µg NCP) compared with control regions. Irreversibly dysfunctional regions (n = 14) had severely reduced perfusion (0.48 ± 0.15 vs. 0.72 ± 0.12 ml/g/min, p < 0.001) and glucose-tracer uptake (52 ± 16 vs. 94 ± 15%, p < 0.001), reduced ATP/ADP ratio (1.5 ± 0.9 vs. 2.3 ± 0.9, p < 0.05), similar glycogen content (579 ± 265 vs. 593 ± 127 nmol/µg NCP) and increased lactate levels (114 ± 52 vs. 89 ± 24 nmol/µg NCP, p < 0.01) compared with control regions.

CONCLUSIONS Contents of metabolic energy stores and lactate in chronic reversibly dysfunctional myocardium were preserved. In contrast, energy stores were depleted in myocardium without functional recovery after revascularization. (J Am Coll Cardiol 2001;37:100–8) © 2001 by the American College of Cardiology

Improvement of myocardial function after coronary artery bypass grafting (CABG) can be predicted by positron emission tomography (PET) in patients with chronic left ventricular dysfunction caused by coronary artery disease (1). Myocardium with chronic reversible dysfunction (“hibernating myocardium”) has normal to moderately reduced perfusion and preserved or increased uptake of the glucose-tracer 18F-fluoro-2-deoxyglucose (FDG), while chronic ir-reversibly dysfunctional myocardium exhibits reduced perfusion and metabolism (1–3). Chronic reversible myocardial dysfunction is associated with episodes of myocardial isch-emia (4,5), but the effect of repetitive ischemic attacks on myocardial metabolism remains unclear (6). The increase in FDG uptake relative to flow in chronic reversibly dysfunc-tional myocardium has been suggested to reflect increased anaerobic glycolysis due to ischemia (7), but the explanation appears unlikely in view of the normal or nearly normal levels of myocardial blood flow (MBF) (2–4,8) and oxygen consumption (8,9). Noninvasive estimates of myocardial perfusion and substrate metabolism achieved by PET may be inaccurate due to partial volume effects (10) and method-ological limitations of the tracer kinetic models (11–13). Increased glycogen stores in chronic reversibly dysfunctional myocardium (4,14–17) may reflect that glucose is diverted away from energy generating pathways toward storage. However, glycogen accumulation is not a consistent finding (18,19), and quantitative analyses on glycogen content are not available.

In this study we analyzed biopsies from dysfunctional myocardium of patients with stable coronary artery disease and left ventricular dysfunction for energy stores and metabolites. Our aims were to study whether chronic reversibly dysfunctional myocardium exhibited any metabolic evidence of ischemia in terms of deranged energy stores and metabolites. We also studied whether myocardial energy stores and metabolites differed between reversibly and irreversibly dysfunctional myocardium. Myocardial glycogen content was measured quantitatively to clarify whether glucose
Abbreviations and Acronyms
CABG = coronary artery bypass grafting
FDG = \(^{18}\)F-fluoro-2-deoxyglucose
MBF = myocardial blood flow
NCP = noncollagen protein
PAS = periodic acid Schiff
PET = positron emission tomography
WMSI = wall motion score index

uptake in chronic dysfunctional myocardium is used for increased storage of glycogen.

METHODS

Patients. We included consecutive patients referred to CABG with left ventricular ejection fraction by ventriculography below 50% and dysfunction in the anterior or lateral left ventricular wall. Patients with myocardial infarction within the last 3 months, aneurysm, unstable angina, diabetes mellitus, previous CABG, cardiac valve disease and congenital heart disease were excluded. The study protocol was approved by the local ethics committee. Informed consent was obtained from all patients.

We included 36 patients, but in three patients biopsies were not available for logistic reasons. In three patients the amount of biopsy material was insufficient for biopsy analysis. From the remaining 30 patients we used the biopsies from 26 patients for analyses of metabolites and biopsies from four patients for a substudy of histology.

Study design. Preoperatively, we performed echocardiography to assess regional wall motion and PET to measure regional myocardial perfusion and FDG uptake. During surgery myocardial biopsies were taken guided by transesophageal echocardiography and from a dysfunctional region in the anterior or lateral left ventricular wall and one from a control region with normal wall motion to eliminate the interindividual variation in myocardial contents of metabolites (20,21). Six months postoperatively we studied graft patency by coronary angiography and wall motion by echocardiography.

Echocardiographic protocol. We obtained echocardiographic images in six standard views (22). Digital echocardiographic recordings and analyses were performed on a Vingmed CFM 750 ultrasonic scanner (Vingmed, Horten, Norway) with a 3.25 MHz transducer connected to a computer equipped with Echopac analysis software (Vingmed, Horten, Norway). Images were digitized on-line (R to R-wave). Regional wall motion scoring was evaluated using the 16-segment model (22). Wall motion score index (WMSI) was calculated in each patient as the sum of segmental scores divided by 16. The initial and follow-up rest studies were displayed side by side in a quad screen digital format and interpreted by an observer blinded to clinical and angiographic data. Improvement of function was defined as improvement by at least one full grade in two adjacent dysfunctional segments on postoperative follow-up. We calculated left ventricular volumes and ejection fraction by the modified Simpson’s method (23) and measured diastolic and systolic wall thickness in biopsy regions on transesophageal and transthoracic echocardiographic images. Systolic wall thickening was calculated as \([(\text{systolic-diastolic wall thickness})/\text{diastolic wall thickness}] \times 100\%\).

PET. Patients were studied during their usual medication. We used whole body PET (Model EXACT HR 961, Siemens/CTI, Knoxville, Tennessee). A 20 min attenuation scan was performed followed by intravenous injection of \(^{13}\)N-ammonia (740 MBq in 20 ml saline) over 30 s with acquisition of a dynamic sequence of images (12 frames of 10 s). For the FDG study patients were given 50 g of oral glucose administered as a 100 ml 50% glucose beverage 1 h before intravenous administration of 370 MBq of FDG over 1 min. Thirty minutes after injection of FDG, three 10-min frames were obtained for semiquantitative measurement of myocardial FDG uptake. Myocardial blood flow in the segments biopsied was calculated as previously described (24). We used diastolic and systolic echocardiographic measurements for assessment of average wall thickness for partial volume correction, assuming an average systolic duration of one-third of the RR-interval. Relative uptake of FDG was assigned to be 100% in the region with the highest MBF. In biopsy regions FDG uptake was expressed as percentages of this activity.

Perioperative myocardial biopsies. From each patient we obtained two transmural myocardial biopsies, one from a dysfunctional region in the anterior or lateral left ventricular wall and one from a control region with normal wall motion. We determined biopsy location before cardiopulmonary bypass by transesophageal echocardiography using a multiplane, two-element annular phased-array 5 MHz transducer (Vingmed, Horten, Norway). After initiation of cardiopulmonary bypass, but before cardioplegia was instituted, biopsies were taken with a 20-mm, 14-gauge Tru-Cut biopsy needle (Baxter Healthcare Corporation, Illinois).

Metabolites in myocardial biopsies. In 26 patients biopsies were frozen in liquid nitrogen within 10 s. We analyzed biopsies for contents of ATP, ADP, AMP, glycogen, lactate and noncollagen protein (NCP) as previously described (21,25–27). Metabolites were related to NCP. All biopsy analyses were performed blindly.

Histological study. We used myocardial biopsies from four patients for histology. Biopsies were immediately fixed in 4% buffered formalin for 1 h, dehydrated in ethanol, embedded in paraffin and cut in 3 \(\mu\)m slices. We stained tissue sections with periodic acid Schiff (PAS) to detect glycogen and performed amylase digestion on an adjacent section followed by PAS staining to verify that the PAS positive material was glycogen. Staining with picrosirius red was performed to detect fibrosis. The histological sections were evaluated qualitatively with regard to glycogen content and degree of fibrosis.

Coronary angiography at follow-up. Graft-patency to biopsy regions were examined by angiography 6 months postoperatively, but 4 of the 30 patients declined coronary
angiography at follow-up. A patent graft was required to have Thrombolysis In Myocardial Infarction (TIMI) grade 3 flow (28).

Statistics. We analyzed data by a two-way repeated measures analysis of variance. We tested for interaction, that is, differences (control and dysfunctional myocardium), in the reversibly dysfunctional group versus differences in the irreversibly dysfunctional group. If this was not significant we tested for main effects. Student t test was used to assess any potential differences between control regions in the two groups. We calculated the 95% confidence intervals for the mean difference between dysfunctional and control regions for each group. Correlation between parameters was tested by least squares linear regression analysis and the correlation coefficient r. We used the statistical software program SPSS 8.0 for statistical analyses. All values are reported as mean ± standard deviation or 95% confidence interval for the mean. A p value < 0.05 was considered statistically significant.

RESULTS

Coronary angiography. We excluded one patient at follow-up because coronary angiography identified an occluded graft to a dysfunctional biopsy region. In the remaining 25 patients with follow-up angiography, all grafts to biopsy regions had TIMI grade 3 flow.

Patients. Preoperative characteristics for the 25 patients in the metabolite and 4 patients in the histological study are shown in Table 1. Patients with and without reversible dysfunction did not differ with regard to mean age, gender, incidence of previous acute myocardial infarction, number of diseased vessels and ejection fraction. Patients with irreversible dysfunction had higher left ventricular end diastolic volumes. This was accompanied by a trend toward higher incidence of Q-waves on the electrocardiogram in this group of patients (p = 0.07). Treatment with long-acting nitrates was more common among patients with reversible dysfunction than those with irreversible dysfunction (11/11 vs. 7/14, p < 0.01). Other medication and risk factors did not differ between groups.

Regional and global left ventricular function (Table 1). At follow-up 6 months after CABG, wall motion had improved in the dysfunctional biopsy region in 11 of the 25 patients in the metabolite study. In spite of reduced systolic wall thickening in reversibly dysfunctional regions compared with control (9 ± 4% vs. 24 ± 8%, p < 0.001), average wall thickness in reversibly dysfunctional regions did not differ from control regions (12 ± 2 mm and 12 ± 2 mm). Irreversibly dysfunctional regions had reduced systolic wall thickening (8 ± 8% vs. 30 ± 14%, p < 0.001) and average wall thickness (8 ± 2 mm vs. 11 ± 2 mm, p < 0.001) compared with control regions.

Ejection fraction did not differ between baseline and follow-up for patients with reversible dysfunction (preoperative 39 ± 7% and postoperative 42 ± 7%, p = 0.11), but WMSI improved from 1.8 ± 0.3 to 1.7 ± 0.2 (p < 0.05).

For patients with irreversible dysfunction, ejection fraction did not differ between baseline and follow-up (preoperative 36 ± 8% and postoperative 33 ± 7%, p = 0.20); however, WMSI decreased from 1.8 ± 0.3 to 1.9 ± 0.3 (p < 0.05).

MBF and FDG uptake. The time from PET to surgery ranged from 4 to 94 days with a median of 14 days. Two patients were not examined by PET. One declined, and another was not studied for logistic reasons. Myocardial blood flow was reduced in reversibly dysfunctional myocardium compared with control regions (0.75 ± 0.19 vs. 0.65 ± 0.16 ml/g/min, p < 0.05). In irreversibly dysfunctional regions MBF was lower than control (0.38 ± 0.08 vs. 0.78 ± 0.16 ml/g/min, p < 0.001) and reversibly dysfunctional regions (p < 0.05). Partial volume correction did not alter the relation between regions (Fig. 1). Correction for differences in the amount of fibrosis between regions, that is, NCP/wet weight, yielded similar estimates for MBF in reversibly dysfunctional regions and control regions (5.39 ± 2.52 and 5.32 ± 1.35 ml/mg NCP/min), whereas irreversibly dysfunctional regions perfusion had reduced perfusion compared with control regions (4.55 ± 1.46 ml/mg NCP/min vs. 6.31 ± 2.09 ml/mg NCP/min, p < 0.01) and reversibly dysfunctional regions (p < 0.05). Relative FDG uptake was similar in control and reversibly dysfunctional regions but reduced in irreversibly dysfunctional myocardium compared with control regions (Fig. 1).

Noncollagen protein (Table 2). A trend towards a lower NCP per wet weight (ww) was observed in reversibly dysfunctional myocardium compared with control regions (p = 0.06). Noncollagen protein/ww in irreversibly dysfunctional myocardium was lower than control (p < 0.05), indicating that the amount of fibrosis was highest in irreversibly dysfunctional regions.

Adenonucleotides (Table 2). Adenonucleotide contents were similar in control regions and myocardium with reversible dysfunction but reduced in irreversibly dysfunctional myocardium. Mitochondrial function estimated by ATP/ADP ratio and energy charge, that is, (ATP + one-half ADP)/(ATP + ADP + AMP), was preserved in myocardium with reversible dysfunction but not in myocardium with irreversible dysfunction.

Glycogen (Table 2). Total glycogen content/NCP was not significantly different between regions. Acid extractable glycogen, which is the most sensitive fraction of glycogen for detection of acute ischemia (29), was preserved in myocardium with reversible dysfunction but reduced in irreversibly dysfunctional myocardium. Content of protein bound glycogen was higher in irreversibly dysfunctional myocardium than it was in control regions. The ratio of acid extractable to protein bound glycogen was reduced in regions of irreversible dysfunction. There was no correlation between total glycogen content and MBF or FDG uptake.

Lactate (Table 2). Lactate content did not differ from control regions in reversibly dysfunctional myocardium. In irreversibly dysfunctional myocardium lactate, content was significantly higher than control. Lactate content in control
### Table 1. Patient Characteristics

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*<p>0.05 versus patients with reversible dysfunction.
AMI = acute myocardial infarction; CCS = Canadian Cardiovascular Society; ECG = electrocardiogram; EF = ejection fraction; LAD = left anterior descending coronary artery; LBBB = left bundle branch block; LCA = left circumflex coronary artery; NA = not available; NYHA = New York Heart Association; RCA = right coronary artery; WM = wall motion.
regions differed between groups and was higher for patients with irreversible dysfunction.

**Histological study** (Fig. 2, A to D). Of the four patients studied two displayed reversible dysfunction on postoperative follow-up. Myocyte morphology was heterogeneous in all regions; myocytes with glycogen accumulation and myocytes appearing normal were seen in control, reversibly and irreversibly dysfunctional myocardium. There was no apparent difference in glycogen content between regions. In irreversibly dysfunctional myocardium the degree of fibrosis was increased.

**DISCUSSION**

The results of this study showed that metabolites and energy stores were preserved in chronic reversibly dysfunctional myocardium in contrast with irreversibly dysfunctional myocardium. Glycogen levels revealed similar total contents in all regions, but the composition of the glycogen pool was altered in chronic irreversibly dysfunctional myocardium.

**Perfusion in dysfunctional myocardium.** It is unknown whether chronic reversibly dysfunctional myocardium is caused by chronic hypoperfusion ("hibernation") or repeti-
interaction of repetitive ischemic episodes separated by periods of vascularization. This observation is compatible with a state of myocardial regions exhibiting functional recovery after reperfusion per se cannot account for the dysfunction observed in regions in irreversibly dysfunctional myocardium. Similar observations have been gained by the use of \( ^{15} \text{O} \)-water PET, which measures average perfusion in the whole myocardial region including scar tissue. However, when we corrected for differences between regions in the degree of fibrosis, measured in biopsies, perfusion did not differ between control regions and chronic reversibly dysfunctional myocardium but remained reduced in irreversibly dysfunctional myocardium. Similar observations have been gained by the use of \( ^{15} \text{O} \)-water PET, which provides values of MBF per gram of perfusable tissue, that is, in the nonfibrotic compartment of the myocardium (2,31). Our findings indicate that abnormal resting perfusion per se cannot account for the dysfunction observed in myocardial regions exhibiting functional recovery after revascularization. This observation is compatible with a state of repetitive ischemic episodes separated by periods of normal perfusion as the cause of contractile dysfunction (4,5).

In one patient MBF was only 0.41 ml/g/min in the control region. However, the distribution of MBF in myocardium with normal contractile function is known to display a wide distribution with values ranging from 0.2 to 2.0 ml/g/min (2,32,33). The average MBF value in control regions in this study is similar to findings by others (33,34).

**Adenonucleotides in chronically dysfunctional myocardium.** Disturbances of myocardial energy metabolism in reversibly dysfunctional myocardium have been suspected due to alterations of the morphology of mitochondria and glycogen accumulation (14,17,19). Flameng et al. (35) found reduced content of adenonucleotides in myocardium supplied by a high-grade stenotic artery without electrocardiographic signs of previous infarction. However, patients were included irrespective of preoperative wall motion score, and no follow-up examination of the patients was performed after revascularization to assess functional outcome in dysfunctional regions. Animal studies show that myocardial high-energy phosphates and ATP/ADP ratio are unaltered in myocardium with reversible dysfunction (36). Noninvasive measurements of ATP performed by \( ^{31} \text{P} \) magnetic resonance spectroscopy in humans (37) also indicate that contents of adenonucleotides are preserved in reversibly dysfunctional myocardium in contrast with irreversibly dysfunctional myocardium. Our findings support that myocytes in chronically dysfunctional regions maintain normal mitochondrial function in contrast with myocytes in irreversibly dysfunctional myocardium.
Myocardial glycogen stores. Morphological studies have demonstrated increased PAS-stained glycogen deposits in chronic reversibly dysfunctional myocardium (4,14,16,17). Activity of glycogen synthase is increased in short-term “hibernating” and repetitively ischemic myocardium (38,39), but glycogen is merely replenished to subnormal levels in these animal studies (38,39). Glycogen accumulation is neither a consistent nor a specific finding in reversibly dysfunctional myocardium (15,18). Shivalkar et al. (19) reported that biopsies from dysfunctional regions with the best postoperative recovery of myocardial function had glycogen content similar to control regions and later confirmed this observation under experimental conditions (36). We used a quantitative method for measurement of myocardial glycogen content and found that total glycogen content was similar in control and reversibly dysfunctional regions. Our findings imply that “supercompensation” of myocardial glycogen stores does not take place in chronic reversibly dysfunctional regions, suggesting that glucose uptake in these regions is used for energy generation.

Nonoxidative glycolysis in reversibly dysfunctional myocardium. Consistent with previous reports (1–3) we found similar FDG uptake in chronic reversibly dysfunctional myocardium and control regions. Although tissue levels of metabolites and energy stores yield no information on metabolic turnover rates (39,40), accumulation of myocardial lactate content reflects anaerobic glycolysis and provides additional information about metabolism in the myocardium. The absence of lactate accumulation in our study does not support that anaerobic glucose metabolism is increased in regions with reversible dysfunction.

Myocardial contents of energy stores and lactate differentiate reversibly from irreversibly dysfunctional myocardium. In irreversibly dysfunctional myocardium, levels of adenonucleotides were reduced and levels of lactate increased. In addition the reduction of acid extractable (i.e., macromolecular) glycogen and increment of protein bound glycogen suggests exposure to ischemia since the macromolecular glycogen pool is most susceptible to degradation during ischemia (29) and is converted into low molecular proglucogen (41,42). Thus, in contrast with reversibly dysfunctional myocardium, metabolism was altered in irreversibly dysfunctional myocardium, in line with magnetic resonance spectroscopic findings (37). This may partly be explained by differences in perfusion because MBF per myocyte appears lower in irreversibly dysfunctional myocardium compared with reversibly dysfunctional myocardium (2,31). It may also be secondary to the more extensive left ventricular dilatation for patients with irreversible dysfunction since remodeling of the left ventricle affects myocardial energy metabolism (43,44). This also explains that anaerobic glycolysis was increased in control regions of patients with irreversible dysfunction due to more extensive left ventricular dilatation.

Figure 2. (A) Periodic acid Schiff stained histological section from control region with normal wall motion. (B) Periodic acid Schiff stained histological section from reversibly dysfunctional myocardium. In both regions myocytes with glycogen accumulation (arrow) and myocytes appearing normal were observed. There was no apparent difference in glycogen content between regions. (C) Biopsy from irreversibly dysfunctional myocardium stained with picrosirius red. The degree of fibrosis (red) is increased, and the amount of myocytes (yellow) is reduced. (D) Biopsy from control region with normal wall motion stained with picrosirius red.
ventricular dilatation and remodeling than in patients with reversible dysfunction (45).

**Clinical implications.** It is unknown whether a state of reversibility can be maintained for an indefinite period of time since a prolonged period of dysfunction may deteriorate myocardial integrity and impair functional outcome (17,46), possibly due to superimposed attacks of severe ischemia. We observed that derangement in metabolism predicted functional outcome after revascularization, but it remains to be studied whether a transition from reversible to irreversible myocardial dysfunction is initiated or accompanied by changes in energy metabolism.

**Study limitations.** The use of NCP as a reference for myocardial content of energy stores may affect the results due to depletion of myocytic protein in dysfunctional regions (4,14–17,19). This error is negligible compared with that introduced using wet or dry weight as reference (27). We used NCP and not dry weight as reference because the amount of connective tissue varies more between regions than the percentage of cells with sarcomere loss (14–16,19).

Different contents of metabolites in the outer and inner layers of the myocardium may be obscured in a transmural biopsy. However, even minor changes in endocardial content of metabolites can be detected in a transmural biopsy (47).

Patients with regional improvement in myocardial function after revascularization had no beneficial effect on global left ventricular function. This observation is similar to the findings by some groups (2) but is in contrast with others (3). Up to 50% of the left ventricle must be dysfunctional and viable before any increase in global left ventricular function can be detected after revascularization (1,48).

**Conclusions.** Contents of metabolic energy stores and lactate in chronic reversibly dysfunctional myocardium were preserved. In contrast, energy stores were depleted in myocardium without functional recovery after revascularization.

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