# Evaluation of Fatty Acid Metabolism in Hearts After Ischemia–Reperfusion Injury Using a Dual-Isotope Autoradiographic Approach and Tissue Assay for Metabolites of Tracer

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We investigated whether changes in myocardial uptake of fatty acid tracer after reperfusion following transient myocardial ischemia were closely related to alterations in intracellular fatty acid oxidation. **Methods:** Using a fatty acid tracer of $^{131}$I- and $^{125}$I-labeled 15-(*p*-iodophenyl)-9-methylpentadecanoic acid (9MPA), the myocardial uptake and metabolites were determined by dual-tracer autoradiography and thin-layer chromatography in rats 3 or 14 d after reperfusion following 5 or 15 min of ischemia induced by coronary artery ligation. **Results:** 9MPA metabolites processed via β-oxidation were lower in the ischemic region (IR) than in non-IR 3 d after 5 min of ischemia, despite no reduction of tracer uptake in IR. Oxidation of 9MPA was recovered 14 d after 15 min of ischemia in association with normalization of tracer uptake in IR, whereas both uptake and oxidation of 9MPA were markedly impaired 3 d after 15 min of ischemia, accompanied by slow clearance of myocardial tracer. **Conclusion:** Normal uptake of fatty acid tracer early after reperfusion does not always imply preserved intracellular fatty acid oxidation. However, reduction of tracer uptake might reflect impaired fatty acid oxidation.

**Key Words:** myocardial ischemia; fatty acid; metabolism; radiotracers

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A branched-chain fatty acid analog such as $^{123}$I-15-β-methyl-*p*-iodophenyl-pentadecanoic acid (BMIPP) has clinically been applied to assess fatty acid metabolism in patients with ischemic heart disease (*1–3*). Abnormal fatty acid metabolism has been estimated by measuring the mismatch in tracer uptake between BMIPP and flow tracer (*2,3*). However, it has not yet been established whether changes in BMIPP uptake are closely related to intracellular oxidation of tracer. In contrast to a straight-chain fatty acid, methyl-branched fatty acid analogs such as BMIPP are expected to interfere with β-oxidation because the metabolic process requires α-oxidation before β-oxidation (*4*). Recently, the radiolabeled fatty acid tracer 15-(*p*-iodophenyl)-9-methylpentadecanoic acid (9MPA) was developed for clinical use (*5,6*). This tracer is converted to an intermediate metabolite of 3-methylnonanoic acid (3MNA) after 3 cycles of β-oxidation, and then α-oxidation is required for further metabolic processes. Therefore, 9MPA is expected to be more feasible for metabolic analyses, including analysis of β-oxidation (*4*). The purpose of the present study was to investigate whether changes in myocardial uptake of 9MPA after reperfusion following transient myocardial ischemia might reflect alterations in intracellular fatty acid oxidation.

## MATERIALS AND METHODS

The present study was undertaken in accordance with the guidelines for animal experimentation at Toyama Medical and Pharmaceutical University.

### Experimental Animals

Fifty-three Wister rats weighing 300–350 g were used for induction of ischemia–reperfusion injury. Myocardial ischemia was produced by ligating the left coronary artery, as described previously (*7*). Briefly, the left coronary artery was ligated 2–3 mm from its origin for 5 or 15 min, and then the ligation was released. The rats were divided into 3 groups according to the duration of ischemia and the recovery period from the ischemia–reperfusion injury. For group A, the metabolic study was performed 3 d after 5 min of coronary ligation; for group B, 3 d after 15 min of ligation; and for group C, 14 d after 15 min of ligation. Cardiac dual-tracer autoradiography was performed to determine myocardial accumulation of fatty acid analog. In separate animals, thin-layer chromatography (TLC) was performed to determine intracellular metabolic products of fatty acid analog. Sham operation

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was performed using the same method as for the ischemia–reperfusion rats, except for the coronary artery ligation.

### Fatty Acid Tracers

In the present study, myocardial fatty acid metabolism was assessed with $^{131}$I- and $^{125}$I-labeled 9MPA (*8*). 9MPA was prepared and supplied by Daiichi Radioisotope Laboratory Co., Ltd. The radiochemical purity of 9MPA was more than 98%, and its specific activity was 30–70 GBq/mmol.

### Cardiac 9MPA Accumulation

Dual-tracer autoradiography with $^{131}$I-9MPA and $^{125}$I-9MPA was performed to evaluate the myocardial uptake and clearance of 9MPA. Animals were injected intravenously with 0.74 MBq of $^{125}$I-9MPA and 55 min later with 5.55 MBq of $^{131}$I-9MPA. The hearts were removed 5 min after the second injection. The specimens were embedded in methyl cellulose and then were prepared as serial 20-μm-thick transverse sections. The first autoradiographic exposure on an imaging plate (BAS-UR; Fuji Film) was performed for 8 h to reveal $^{131}$I-9MPA accumulation. The second exposure, for $^{125}$I-9MPA imaging, was initiated 75 d later, after the decay of $^{131}$I-9MPA activity, and required 30 d for adequate image quality. For the present doses of $^{131}$I and $^{125}$I, cross-talk between the 2 tracers was less than 3%, and cross-talk between $^{131}$I and $^{125}$I could therefore be negligible.

To determine the myocardial accumulation of 9MPA, the autoradiographic images were analyzed using a computer-assisted image-processing system (BAS3000; Fuji Film), as described previously (*9*). Regions of interest were put on the left ventricular anterior wall (ischemic region, or IR) and septal wall (non-IR) at the level of the papillary muscles, and IR was defined as one sixth of the whole left ventricular area around the center of ischemia on the autoradiographic image. Myocardial tracer uptake in IR and non-IR was normalized as a percentage of the administered dose per gram of heart (% dose/g), using $^{131}$I- and $^{125}$I-labeled graded standards. Clearance of 9MPA, that is, washout rate, in the ischemic and nonischemic segments was calculated using the following equation:

$$\text{Washout rate (\%)} = (^{131}\text{I uptake} - {}^{125}\text{I uptake}) \times 100/{}^{131}\text{I uptake}.$$

### Analysis of 9MPA Metabolites

Lipids were extracted from myocardial tissues according to a modification of the method of Folch et al. (*10*), with metabolic products of 9MPA being assessed by TLC. A dose of 3.7 MBq of $^{125}$I-9MPA was administered intravenously while the animal was receiving pentobarbital anesthesia. The hearts were quickly removed 5 min after the injection. The left ventricular tissues of IR and non-IR were separately homogenized. The radioactivity of 9MPA metabolites was assayed by TLC on aluminum sheets (RP-$^{18}$F; Merck) in conjunction with standard lipid preparations. Metabolites of 9MPA on the aluminum sheets were exposed on the imaging plate for 14 d and quantified with a bioimaging analyzer (BAS3000). Two major metabolites of 9MPA detected on the exposed images were 3MNA, as the intermediate metabolite after 3 cycles of β-oxidation from 9MPA, and *p*-iodo-phenyl acetic acid, representing the final product of 9MPA (*8*).

### Statistical Analysis

Data are expressed as mean ± SD. Group comparisons were made with ANOVA, followed by a Bonferroni test to identify differences among various groups. $P < 0.05$ was considered statistically significant.

## RESULTS

### Cardiac 9MPA Uptake

As shown in Figure 1 and Table 1, both $^{131}$I and $^{125}$I tracer accumulation in IR were not different from those in non-IR in group A. In group B, however, $^{131}$I-tracer accumulation in IR reduced significantly, but $^{125}$I-tracer accumulation was not different between these regions. Consequently, tracer cleared more slowly from IR than from non-IR. The reduced tracer uptake in IR recovered 14 d later (group C). The uptake ratio of IR to non-IR was decreased only in the $^{131}$I image of group B (Fig. 2).

### Thin-Layer Chromatography

Representative examples of TLC in IR and non-IR are shown in Figure 3. The sum of 3MNA, *p*-iodo-phenyl acetic acid, and the other intermediate metabolites processed via β-oxidation was defined as the 9MPA metabolites in the present study. The 9MPA metabolites in group A were significantly lower in IR than in non-IR despite the equivalent tracer accumulation between these regions (Table 2; Fig. 4). 9MPA metabolites of IR were markedly reduced in group B but recovered in group C.

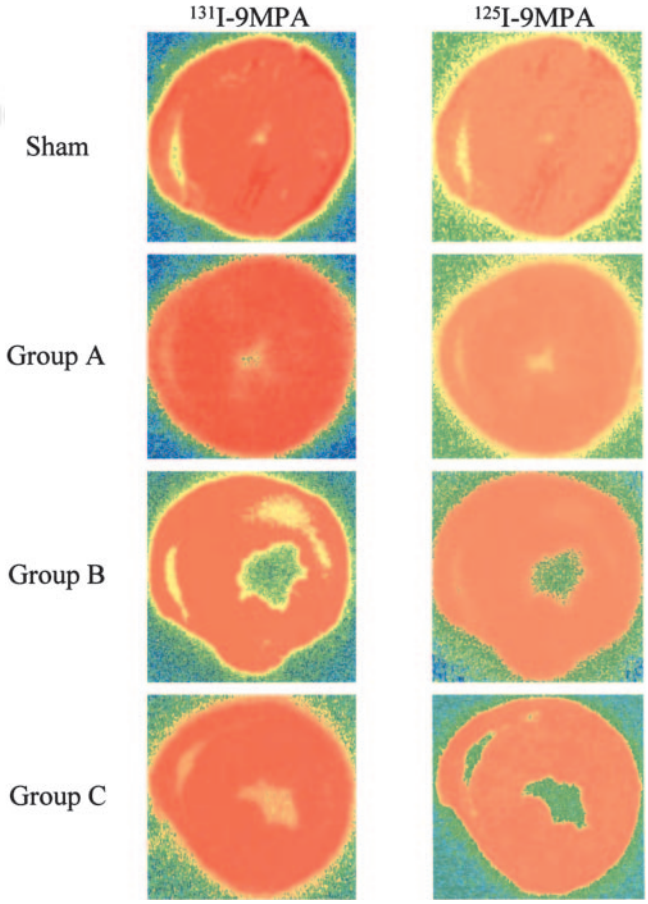


**FIGURE 1.** Representative examples of myocardial 9MPA uptake shown by dual-tracer autoradiography. $^{131}$I-9MPA represents image 5 min after tracer injection, and $^{125}$I-9MPA, 60 min after injection.

**TABLE 1**
Myocardial 9MPA Uptake and Washout Rate

| Group | $^{131}$I (% dose/g) | | $^{125}$I (% dose/g) | | Washout rate (%) | |
|---|---|---|---|---|---|---|
| | IR | Non-IR | IR | Non-IR | IR | Non-IR |
| Sham ($n$ = 5) | 6.32 ± 1.03 | 6.69 ± 1.17 | 3.12 ± 0.65 | 3.20 ± 0.65 | 50.7 ± 5.6 | 52.2 ± 4.9 |
| A ($n$ = 6) | 5.49 ± 1.02 | 5.57 ± 1.16 | 3.00 ± 0.74 | 3.16 ± 0.94 | 45.1 ± 11.8 | 43.5 ± 11.1 |
| B ($n$ = 5) | 5.23 ± 1.10* | 7.52 ± 0.73 | 3.13 ± 0.27 | 3.47 ± 0.50 | 37.4 ± 16.9† | 53.6 ± 7.8 |
| C ($n$ = 6) | 6.21 ± 0.94 | 6.48 ± 1.50 | 2.83 ± 0.32 | 2.82 ± 0.45 | 53.7 ± 8.0 | 55.3 ± 9.2 |

*$P < 0.01$ vs. non-IR.
†$P < 0.05$ vs. non-IR.
Washout rate of 9MPA = ($^{131}$I uptake − $^{125}$I uptake) × 100/$^{131}$I uptake.

## DISCUSSION

The major findings of the present study were as follows. First, abnormal myocardial 9MPA oxidation was present 3 d after reperfusion following 5 min of ischemia despite its normal accumulation. Thus, a lack of reduction in fatty acid tracer uptake early after ischemia does not always imply normal intracellular fatty acid oxidation. Second, the reduced tracer uptake seen in the early image after tracer injection was associated with impaired fatty acid oxidation and was not present in the delayed image because of slow clearance of tracer. Third, normalization of reduced tracer uptake after ischemia–reperfusion injury was accompanied by recovery of impaired fatty acid oxidation.

### 9MPA Accumulation

Long-chain fatty acids are a major energy substrate for normoxic myocardium. In ischemia, glucose plays a major role in energy production, whereas oxidation of long-chain fatty acids is inhibited. Thus, an alteration of fatty acid metabolism is considered to be a sensitive marker of ischemia. In patients with acute coronary syndrome who had undergone direct percutaneous transluminal coronary angioplasty, 9MPA images obtained within 2 wk after the onset revealed lower accumulation and slower clearance of 9MPA in IR than in non-IR (*6*). Similar findings were reported for patients with angina pectoris and myocardial infarction (*5*). These observations were consistent with the present results. In experimental models of myocardial ischemia–reperfusion, however, greater uptake of fatty acid tracer than of $^{201}$Tl chloride was reported (*11,12*). The conflict data between these studies may be explained by the differences in time from the reperfusion to the metabolic study; that is, the fatty acid tracer study was performed early after reperfusion in the latter. In the acute stage after reperfusion, the prolonged residence of fatty acid tracer due to increased triglyceride pool and interstitial lipid accumulation may cause greater retention of the tracer, despite the increased backdiffusion (*11*). In the chronic stage, however, tracer extraction may decrease along with an increase in backdiffusion (*4*). Most clinical studies using BMIPP (*2,3*) or 9MPA (*5,6*) showed lower uptake of fatty acid tracer than of flow tracer in IR. Slow recovery of fatty acid tracer uptake in the present study is also consistent with the clinical study (*13*).

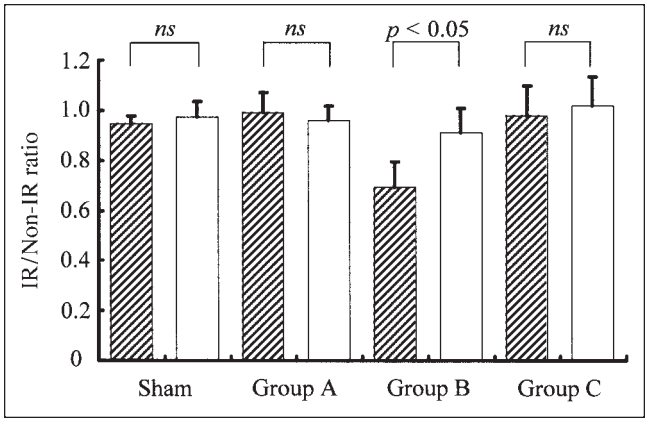


**FIGURE 2.** 9MPA uptake ratio of IR to non-IR in sham-operated rats ($n$ = 5) and rats of groups A ($n$ = 6), B ($n$ = 5), and C ($n$ = 6). Hatched bars indicate $^{131}$I-9MPA uptake ratio, and open bars, $^{125}$I-9MPA uptake ratio. ns = not statistically significant.

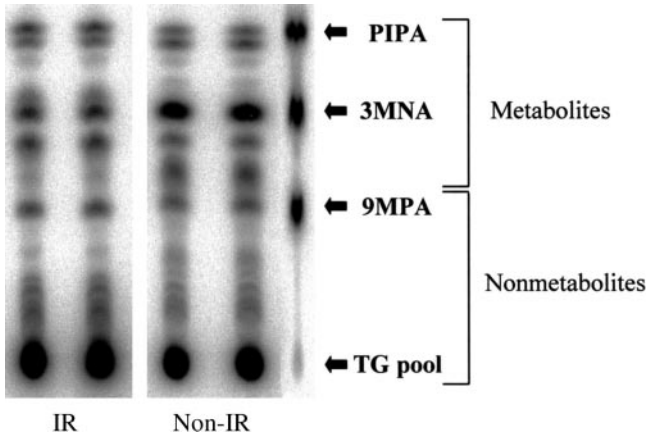


**FIGURE 3.** Representative examples of TLC of 9MPA in a rat 3 d after reperfusion following a 15-min coronary occlusion. Bottom spots are 9MPA in triglyceride (TG) pool. Sums of radioactivity from intermediate metabolites above 9MPA to *p*-iodo-phenyl acetic acid (PIPA) were defined as metabolites processed by β-oxidation.

**TABLE 2**
9MPA Metabolites and Nonmetabolites in IR and Non-IR

| Group | Nonmetabolites (%) | | Metabolites (%) | |
|---|---|---|---|---|
| | IR | Non-IR | IR | Non-IR |
| Sham ($n$ = 5) | 34.4 ± 5.9 | 34.0 ± 3.2 | 65.6 ± 5.9 | 66.0 ± 3.2 |
| A ($n$ = 8) | 50.9 ± 5.6* | 42.2 ± 4.7 | 49.6 ± 6.1* | 58.1 ± 5.1 |
| B ($n$ = 9) | 61.9 ± 9.7* | 46.1 ± 1.1 | 38.1 ± 9.7* | 53.8 ± 1.1 |
| C ($n$ = 9) | 38.8 ± 7.1 | 34.7 ± 6.4 | 61.1 ± 7.1 | 65.2 ± 6.4 |

*$P < 0.01$ vs. non-IR.
Nonmetabolites indicate 9MPA before β-oxidation and metabolites indicate 9MPA processed by β-oxidation.

$^{131}$I-9MPA uptake in non-IR was greater in group B than in group A, although uptake in group B was significantly lower in IR than in non-IR (Table 1). We do not have a plausible explanation for this finding, but increased sympathetic nerve activity and a concomitant increase in plasma levels of free fatty acid because of relatively prolonged ischemia could possibly contribute to this increased fatty acid uptake 3 d after the reperfusion.

### Fatty Acid Metabolism

Metabolic processing of fatty acid analogs with a methyl branch may be limited in the heart, whereas straight-chain fatty acids would be metabolized rapidly by β-oxidation (*4*). Recent animal studies (*14,15*), however, demonstrated that β-oxidation metabolites of BMIPP were detected in coronary venous samples and the amount of β-oxidation metabolites was affected by changes in energy substrate and myocardial disorder. Unfortunately, the intracellular contents of BMIPP metabolites were not evaluated in these studies. The tracer of 9MPA is converted to 3MNA after 3 cycles of β-oxidation, and α-oxidation is required for further metabolic processes. Therefore, 9MPA is expected to be washed out from myocardium at medium rates; that is, it is cleared more slowly than are straight-chain fatty acids but more quickly than is BMIPP (*4*).

The discrepancy between myocardial tracer uptake and oxidation found in group A of the present study is consistent with that found by Chandler et al. (*16*), who showed that myocardial fatty acid uptake was not affected despite a decrease of fatty acid oxidation in demand-induced ischemia, suggesting that there was greater conversion of fatty acids to intracardiac triglyceride stores. The metabolic alterations persisted at least 3 d after reperfusion in the present study. In a dog experiment of 1 h of ischemia followed by reperfusion, fatty acid metabolism, as assessed by clearance of $^{11}$C-palmitic acid, was impaired for a prolonged time but recovered at 1 wk after the reperfusion along with parallel improvement of regional ventricular function (*17*). A similar result was reported for 3 h of ischemia, but the time course for recovery was longer (*18*). In contrast, quick recovery of fatty acid metabolism after reperfusion was reported (*19,20*). These findings may have resulted, at least in part, from the differences in time from reperfusion; that is, the latter studies were performed early after reperfusion.

### Limitations

Some methodologic limitations deserve comment. First, regional myocardial flow was not measured in the present study. In our preliminary study using $^{201}$Tl chloride, the uptake ratio of IR to remote regions was mildly decreased 3 d after reperfusion following 15 min of ischemia (0.75 ± 017, $n$ = 4) and recovered 7 d later (0.90 ± 0.08, $n$ = 5). Reduced uptake of 9MPA 3 d after reperfusion in the present study might partially be affected by impaired microcirculation, but the reduced 9MPA metabolites induced by reperfusion following 15 min of ischemia could derive primarily from impaired fatty acid oxidation.

Second, IR was not precisely determined during the coronary artery ligation but was determined by our previous study of rat myocardial infarction (*7*). In the present study, IR was determined as the region distal to the occlusion site in the TLC study and the region including one sixth of left ventricular area in the short-axis slice at the level of papillary muscle on dual-tracer autoradiography. Therefore, the ischemic area selected in the present study might be included within the ischemic myocardium induced by the coronary artery ligation.

## CONCLUSION

This study indicated that normal tracer uptake early after reperfusion following transient myocardial ischemia may not always imply unimpaired intracellular fatty acid oxidation, and careful attention should be paid to the interpretation of images of fatty acid tracer, especially of 9MPA. However, reduced tracer uptake suggests impaired fatty acid oxidation, and analysis of dynamic images early after the tracer injection may allow detection of impaired fatty acid metabolism.

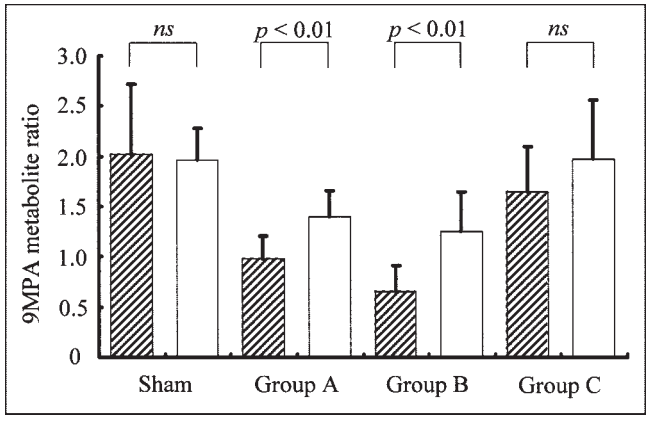


**FIGURE 4.** Ratio of 9MPA metabolites processed by β-oxidation to 9MPA nonmetabolites. Hatched bars indicate ratio obtained from IR, and open bars, from non-IR, in sham-operated rats ($n$ = 5) and rats of groups A ($n$ = 8), B ($n$ = 9), and C ($n$ = 9). ns = not statistically significant.

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