

Myocardial Metabolism of ^{123}I -BMIPP in a Canine Model with Ischemia: Implications of Perfusion–Metabolism Mismatch on SPECT Images in Patients with Ischemic Heart Disease

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^{123}I -(*p*-iodophenyl)-3-*R,S*-methylpentadecanoic acid (BMIPP) is a fatty acid analog for SPECT imaging. This radiopharmaceutical possesses the unique property, that is, perfusion–metabolism mismatch on SPECT images in patients with ischemic heart disease. However, the reason of this mechanism remains unclear. **Methods:** Using open-chest dogs under anesthesia, we made a system to release all the blood of the great cardiac vein outside without recirculation, if necessary. Left anterior descending artery (LAD) was occluded for 30 min after reperfusion. After the injection of BMIPP into LAD, blood samplings from the cardiac vein and abdominal aorta (6 dogs) or serial biopsy specimens from the LAD region (5 dogs) were performed, and then compared with the normal control. The catabolites of BMIPP, including backdiffusion of nonmetabolized BMIPP, were evaluated with high-performance liquid chromatography (HPLC) in the efflux study. Thin-layer chromatography (TLC) technique was introduced in the tissue analytical study. **Results:** Although the rapid extraction of BMIPP from the plasma into the myocardium and the subsequent retention were unchanged, the early washout (8 min) of radioactivity significantly increased ($51\% \pm 12\%$ to $65\% \pm 7\%$; $P < 0.05$) with ischemia. The metabolites from the myocardium consisted of backdiffusion of nonmetabolized BMIPP, α , intermediate, and full oxidation metabolites. Among these metabolites, backdiffusion of nonmetabolized BMIPP in blood significantly increased ($27.9\% \pm 7.7\%$ to $42.3\% \pm 8.1\%$; $P < 0.05$), especially in the early phase with ischemia. In tissue, the radioactivity was concentrated in the triglyceride pool even in the early phase, and in addition, BMIPP and α -oxidized metabolite significantly decreased in the early phase with ischemia ($t = 1$ min after BMIPP injection, $25.9\% \pm 8.6\%$ to $14.5\% \pm 2.1\%$, $P < 0.01$; $t = 2$ min, $8.9\% \pm 5.0\%$ to $4.5\% \pm 1.7\%$, $P < 0.05$). **Conclusion:** These results show that backdiffusion of nonmetabolized BMIPP from the myocardium increased and BMIPP (long-chain fatty acids) in tissue decreased with ischemia, suggesting backdiffusion of nonmetabolized BMIPP might play an important role in myocardial perfusion–metabolism mismatch on SPECT images in patients with ischemic heart disease.

Key Words: ^{123}I -BMIPP; mismatch; ischemic heart disease; backdiffusion

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Naturally occurring, long-chain fatty acids such as palmitic acid are the major energy source for the normoxic myocardium, and thus, radioiodinated fatty acid agents represent potential probes to evaluate differences in cardiac oxidative metabolism that are present in various myocardial disorders. However, straight-chain fatty acids are rapidly metabolized by β -oxidation, and thus, structural modifications have been proposed as a method of improving image quality. ^{123}I -(*p*-iodophenyl)-3-*R,S*-methylpentadecanoic acid (BMIPP) is a radioiodinated fatty acid analog with a methyl branch at the β 3 position. This structure of BMIPP inhibits rapid myocardial catabolism (1,2), and thus, BMIPP has a long retention in the myocardium by incorporation into the triglyceride pool (3,4), showing good characteristics for clinical SPECT imaging. Many clinical protocols that use BMIPP have been performed in both Japan and western Europe, and several studies have reported that BMIPP possesses the unique property of perfusion–metabolism mismatch, that is, decreased BMIPP uptake with relatively preserved myocardial perfusion, on SPECT images in patients with ischemic heart disease (5–8).

Our previous study, which used an animal model, demonstrated that backdiffusion of BMIPP significantly increased in the early phase; the full metabolite of β -oxidation in the late phase also decreased from the dysfunctional myocardium created by etomoxir, a carnitine shuttle inhibitor; and in addition, total backdiffusion of BMIPP was significant (9). Our recent study also elucidated that backdiffusion of nonmetabolized BMIPP increased and full metabolite of complete β -oxidation decreased in proportion to the severity of ischemia with an occlusion and reperfusion canine model

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(10). However, the mechanism of perfusion–metabolism mismatch remains to be elucidated.

The purpose of this study was to examine the myocardial metabolism of BMIPP both in efflux of the blood and in tissue after regional ischemia and reperfusion, trying to explain the mechanism of perfusion–metabolism mismatch on SPECT images in patients with ischemic heart disease.

This study initially elucidated the mechanism of “mismatch” from the analyses both in efflux and in tissue with an animal model.

MATERIALS AND METHODS

This study followed the Guidelines for Animal Experiments of Kyoto University established in 1988.

After an overnight fast, adult male mongrel dogs weighing 21.0–29.5 kg were anesthetized by intramuscular injection of ketalar (2.5 mg/kg) for the induction, followed by intravenous injection of pentobarbital (25 mg/kg) for the maintenance of anesthesia. After endotracheal intubation, the animals were connected to a dual-phase control respirator (Harvard Apparatus, Southnatic, MA) supplying 100% oxygen at 2 L/min. A catheter was inserted into one femoral artery to monitor blood pressure, and another catheter for arterial blood sampling was inserted into the abdominal aorta through the opposite femoral artery. A triple-lumen intravenous catheter was placed in the femoral vein for fluid infusion and drug administration. Thoracotomy was performed at the fifth intercostal space, and the epicardium was fixed to the thoracic wall in the form of a cradle. The left anterior descending artery (LAD) was dissected free for radioisotope administration, and a Doppler flow probe and a snare were implanted around the proximal part of the LAD. As shown in Figure 1, the great cardiac vein (GCV) was also dissected free, cannulated, and a three-way valve was attached to switch the blood flow to the left appendage of the heart for recirculation or to the open port for venous blood sampling. The colored microspheres were infused through the catheter into the left appendage for the measurement of regional myocardial blood flow (MBF). The catheter was later used for the injection of Evans Blue for the calculation of ischemic region. The wall tracker module that was used for ultrasonography (WT-10; Crystal Biotech, Hopkinton, MA) was attached to the LAD region of the left ventricle for the evaluation of regional wall thickening (Fig. 1).

Experimental Protocol

The experimental protocol is summarized in Figure 2. Surgical procedures were performed on 39 dogs. Twelve dogs died during surgery or procedures of the protocols despite resuscitation and were thus excluded from the study. Another 4 dogs were excluded from this study, 3 dogs because of acute myocardial infarction and 2 dogs because of inadequately decreased MBF during ischemia.

The remaining 23 dogs completed the protocol. Animals were divided into four groups: the control group of efflux study (the control efflux group, $n = 6$), the group of efflux study on ischemia (the ischemia efflux group, $n = 6$), the control group with tissue biopsy (the control biopsy group, $n = 6$) and the group with tissue biopsy on ischemia (the ischemia biopsy group, $n = 5$). In the efflux study on ischemia, 30-min LAD occlusion, followed by 20 min of reperfusion, was performed, and then the extraction, retention and metabolism studies were completed. In the biopsy study on ischemia, after 30-min LAD occlusion, followed by 20

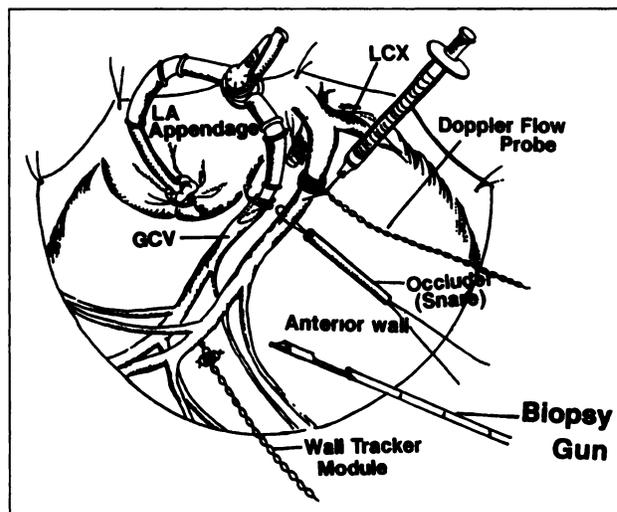


FIGURE 1. Illustration of experimental procedure on canine heart. Doppler flow probe and snare were implanted around proximal part of LAD. Wall tracker module was attached to left ventricular wall where LAD dominated in efflux study. Biopsy gun was used for tissue samplings in biopsy study. LCX = left circumflex artery; GCV = great cardiac vein; LA = left atrium.

min of reperfusion, the biopsy of serial tissue samplings were performed in the same way as the control.

Measurement of Regional Myocardial Blood Flow

MBF was measured four times with colored microspheres: at baseline, during LAD occlusion, after 20 min of reperfusion and the end of the protocol in the efflux study (Fig. 2). Red, yellow, blue and black colored microspheres were chosen in this study. In the biopsy study with ischemia, MBF was measured only at baseline and during LAD occlusion to validate the myocardial flow reduction. The colored microspheres were nonradioactive (E-Z Co. Ltd., Los Angeles, CA) and 7–10 million microspheres were injected into the left atrium with a syringe after sufficient manual mixing with another connected syringe. Concomitantly, blood samples were collected from the femoral artery at a rate of 10 mL/min by 90-s aspiration. After the dogs were killed, myocardial tissue samples (each piece weighing 0.5–1.0 g) were collected from 20 sites on the epicardial and endocardial layers of each ischemic and normal area. Each sample was then treated by routine methods, as previously reported (11). Microspheres were counted under a microscope at $\times 100$ –400 magnification, and regional MBF was quantified. Evans Blue and triphenyl tetrazolium chloride (TTC) stains did not affect this measurement.

Measurement of Myocardial Thickening

Myocardial thickening was assessed with a wall tracker module (WT-10; Crystal Biotech), as described in a previous report (12). The beginning and end of the systolic interval were determined from the onset of the initial uptake of left ventricular dP/dt and 20 ms before peak negative dP/dt , respectively. Regional left ventricular wall function was estimated as net systolic thickening. The thickening fraction was calculated by dividing the transmural net systolic thickening by the end diastolic wall thickness.

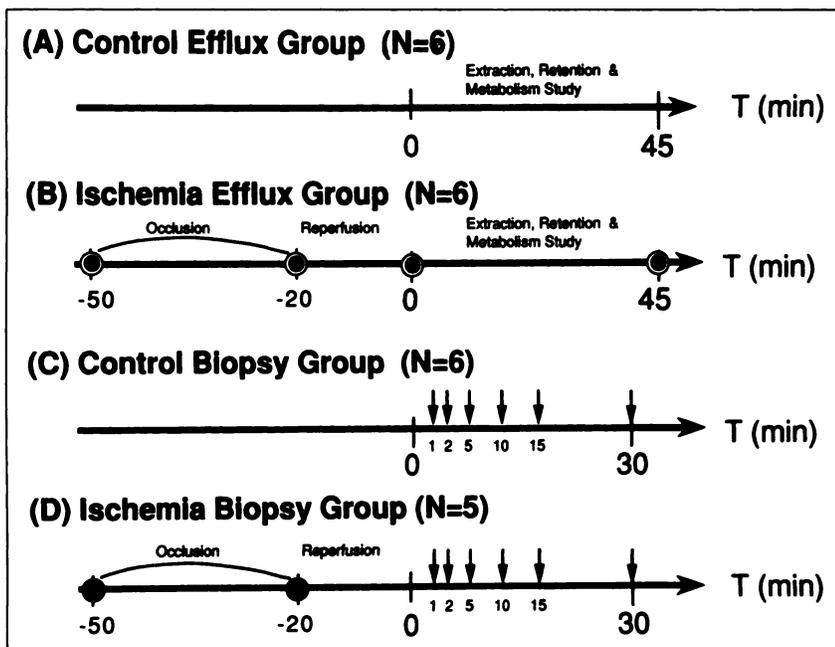


FIGURE 2. Illustration of experimental time schedule. Starting times of efflux studies or biopsy studies were defined as zero (min). ● = measurement of microspheres blood flow, ○ = measurement of wall thickening, oxygen saturation and lactate; ↓ = performance of tissue needle biopsy from LAD region.

Measurement of Metabolic Parameters

The blood sampling from the GCV and the abdominal aorta were performed to measure lactate and to estimate the ischemic condition. Lactate consumption was calculated as follows:

$$\text{lactate consumption} = \frac{\text{arterial lactate (mg/dl)} - \text{venous lactate (mg/dl)}}{\text{arterial lactate (mg/dl)}} \times 100 (\%).$$

The oxygen saturation of blood in the GCV or the abdominal aorta was continuously monitored by OXIMETRIX 3 (Abbot Laboratories, Abbot Park, IL), and the change in the difference between venous blood and arterial blood values was considered to represent ischemia or flow reduction.

Evaluation of Ischemic Size

Just before the dogs were killed, Evans blue dye (1.0%, 100 mL) was injected through the left appendage, after the complete occlusion of the LAD with a snare. The stained left ventricle was then sectioned into 4–5 short axial slices of equal thickness (1–1.5 cm) from base to apex to evaluate the ischemic area. The stained and nonstained areas were traced, and the ischemic areas were calculated as a percentage of the whole cut surface on the both sides of each slice and were averaged and multiplied by the weight of the slice. All the ischemic sizes were summed up and expressed as a percentage of the left ventricular size.

Evaluation of Infarct Area

All slices were stained with 1%, 37°C TTC for 20 min to detect the infarct area. Dogs having shown apparent infarction were excluded from the study.

Extraction of BMIPP

This study followed our previous protocol (13) and involved collection of all the blood from the GCV for 60 s just after the injection of a mixture of ^{123}I -BMIPP (0.5 μCi) and ^{125}I -bovine serum albumin (0.5 μCi) in 100 μL saline. The collected blood samples were weighed, and the radioactivity was measured by a well scintillation counter (ARC-350; ALOKA, Tokyo, Japan) with decay correction. The actual radioactive content of ^{123}I and ^{125}I in

the samples was calculated with the crosstalk ratio obtained from the ^{123}I standard sample (crosstalk from ^{125}I to ^{123}I was negligible). The average flow rate of the GCV was calculated from the weight of the blood samples and the extraction fraction as follows:

Extraction fraction =

$$1 - \frac{[^{123}\text{I} \text{ in the blood (0–30 s)}] / [^{123}\text{I} \text{ injected}]}{[^{125}\text{I} \text{ in the blood (0–30 s)}] / [^{125}\text{I} \text{ injected}]}$$

Retention and Metabolism of BMIPP in Efflux Study

Just after the extraction study, BMIPP (^{123}I : 2 mCi, 0.2 mL) was injected into the LAD, and both venous blood from the GCV and arterial blood from the abdominal aorta were collected into heparinized tubes at various time intervals (30 s, 1 min, 2 min, 5 min, 10 min, 15 min and 30 min after injection). Plasma samples were separated by centrifugation at 3000 rpm for 10 min, and the radioactivity of a 0.1-mL aliquot was measured by a well scintillation counter as soon as possible. The remainder of the plasma was extracted twice with a 2:1 mixture of chloroform and methanol (14). The organic layer was then collected and evaporated, and the residue dissolved in 500 μL methanol for high-performance liquid chromatography (HPLC) analysis. An LC-6A chromatographic system (Shimadzu Co. Ltd., Kyoto, Japan) with a YMC-Pack ODS Column (20 \times 150 + 20 \times 50 mm, YMC Co. Ltd., Kyoto, Japan) were used for the HPLC analysis. The mobile phase was methanol: water:acetic acid (96:4:1) with a flow rate of 6 mL/min. After injection of the sample, the eluate was collected in 1-min fractions with a fraction collector. The radioactivity of each fraction was then measured with the well scintillation counter with decay correction.

Data Calculation in Efflux Study

The area under the curve (AUC) of time-activity data were calculated with trapezoid algorithm. The following parameters were also calculated:

1. Cumulative dose = injected dose \times extraction fraction.
2. Washout dose (0.5–30 min) = AUC of (radioactivity in GCV

plasma – radioactivity in arterial plasma)

$$\times \text{average flow rate of GCV} \times \frac{100 - \text{hematocrit}}{100}.$$

3. Retention fraction at 30 min = 1 – washout dose/cumulative dose.

4. % washout in the early phase (8 min)

$$= \frac{\text{Washout dose (8 min)}}{\text{Washout dose (30 min)}} \times 100.$$

Also calculated was the percentage cumulative metabolite washout fraction (0.5–30 min).

From the total radioactivity in plasma and the fraction of each metabolite obtained by HPLC, the plasma metabolite levels were calculated. Washout of each metabolite from the myocardium was then estimated from the difference in the metabolite levels of arterial and GCV plasma. The extraction of BMIPP from the arterial plasma was taken into consideration as follows:

$$\text{Washout of BMIPP} = \text{GCV content} - \text{arterial content} \\ \times (1 - \text{extraction fraction}).$$

The AUC of metabolite washout was calculated with trapezoid algorithm. The cumulative metabolite washout fraction (1–30 min) was then calculated as follows:

$$\text{Cumulative metabolite washout fraction} \\ = \text{AUC of metabolite} / \text{sum of each metabolite AUC}.$$

Metabolism of BMIPP in Biopsy Study

In the biopsy studies, ^{123}I -BMIPP (2 mCi, 0.2 mL) was injected into the LAD, and the tissue needle biopsy of the LAD region was performed transmurally at 1, 2, 5, 10, 15 and 30 min after injection of radioisotope.

The tissue samples were then rinsed in a cold saline solution for 3–5 s to clear the tissue of blood, and these samples were frozen in ceramic bowls as soon as possible with liquid nitrogen. Each sample was then ground down with a pestle and was extracted with a 2:1 mixture of chloroform and methanol (14) immediately after the freezing procedure. An aliquot of the extract was chromatographed on thin-layer chromatography (TLC) plates (Silica gel-60; E Merck, Darmstadt, Germany) using a solvent system: n-hexane:diethyleter:acetic acid (60:40:1), as previously reported (15). The radioactivity was measured by a well scintillation counter. The radio-peaks were identified by a comparison with the standard samples.

Statistical Analysis

All measured values were analyzed with Student *t* test, and serial changes with the analysis of variance (ANOVA) program. A value was expressed in terms of mean \pm SD, and a value of $P < 0.05$ was considered statistically significant.

RESULTS

Hemodynamics

There was no significant change in either heart rate or aortic blood pressure during all the experiments.

Regional Myocardial Blood Flow

MBF during the experiments with ischemia are summarized in Table 1. The MBF in the ischemic region was

expressed as an absolute value and a percentage of the baseline control.

The percentage MBF in efflux study significantly decreased to $28.0\% \pm 17.5\%$ during occlusion ($P < 0.001$). However, percentage MBF after 20 min of reperfusion and at the end of the protocol did not change significantly.

The percentage MBF in biopsy study also significantly decreased to $17.6\% \pm 13.7\%$ during occlusion ($P < 0.001$), and this result validated myocardial flow reduction during occlusion in biopsy study. These two values were slightly different but did not achieve statistical significance.

Regional Myocardial Thickening

The changes in the thickening fraction were examined during the ischemia efflux study, and myocardial thickening in the LAD region was expressed as a percentage of the baseline control. The percentage myocardial thickening significantly decreased to $-34.6\% \pm 13.1\%$ ($P < 0.001$ versus baseline) during ischemia. These values also significantly decreased after reperfusion ($9.8\% \pm 8.4\%$; $P < 0.001$ versus baseline) and at the end of the protocol ($18.7\% \pm 13.4\%$; $P < 0.001$ versus baseline).

Metabolic Parameters

Lactate consumption and oxygen saturation were measured in the efflux study with ischemia (Table 2). Lactate consumption during occlusion significantly decreased from $33.5\% \pm 10.1\%$ to $-16.5\% \pm 40.8\%$ ($P < 0.01$) compared with the baseline control; however, these values after reperfusion and at the end of the protocol did not change significantly. Arterial-venous difference of oxygen saturation significantly increased from $77.0\% \pm 3.8\%$ to $82.2\% \pm 4.5\%$ ($P < 0.05$) during occlusion compared with the baseline, which represented ischemia or flow reduction because hemoglobin levels did not change during experimental protocols.

Ischemic Sizes

The ischemic areas in the LAD region were certified in all canine hearts and were used for the experiments with ischemia. The percentage of ischemic areas (the size of area at risk) was $22.5\% \pm 4.7\%$ (the ischemia efflux group) and $21.9\% \pm 5.2\%$ (the ischemia biopsy group). There was no significant difference between these two groups.

Infarct Area by Triphenyl Tetrazolium Chloride Staining

The apparent infarct areas by TTC staining in the LAD region were certified in 3 of 39 dogs used for the experiments. These 3 dogs were excluded from the study.

Metabolism of BMIPP in Efflux Study

Extraction, retention and percentage washout in the early phase are shown in Table 3. Although extraction (30 s) and retention (30 min) did not change significantly with ischemia, the percentage washout in the early phase (8 min) significantly increased from $51\% \pm 12\%$ to $65\% \pm 7\%$ ($P < 0.05$) in ischemia.

TABLE 1
Regional Myocardial Blood Flow in Ischemic Area and Normal Control

	Flow (mL/min × g)			
	Baseline	Occlusion	Reperfusion	Final
Efflux study				
Ischemic area of LAD occlusion	0.87 ± 0.27	0.22 ± 0.10*	0.76 ± 0.11	0.87 ± 0.32
Normal control	0.82 ± 0.20	0.99 ± 0.31	0.86 ± 0.33	0.92 ± 0.44
Biopsy study				
Ischemic area of LAD occlusion	0.99 ± 0.16	0.16 ± 0.08*		
Normal control	1.03 ± 0.25	1.10 ± 0.30		

	Flow (% Baseline control)			
	Baseline	Occlusion	Reperfusion	Final
Efflux study				
Ischemic area of LAD occlusion	100	28.0 ± 17.5*	89.6 ± 25.6	96.4 ± 30.9
Normal control	100	123.1 ± 34.2	103.9 ± 27.9	105.5 ± 37.5
Biopsy study				
Ischemic area of LAD occlusion	100	17.6 ± 13.7*		
Normal control	100	107.3 ± 11.7		

**P* < 0.001 compared with baseline.
LAD = Left anterior descending artery.

A typical HPLC illustrated the identification of four major peaks, and the metabolites from the myocardium were divided into the following four groups: the full-oxidation metabolite of BMIPP (2-(*p*-iodophenyl) acetic acid), the intermediate metabolites, the α -oxidation metabolite (14-(*p*-iodophenyl)-2(α)-R,S-methyltetradecanoic acid) and the backdiffusion of nonmetabolized BMIPP, as previously reported (9,10,13). The washout of each metabolite from the myocardium was calculated from these data, and the cumulative washout fraction of each metabolite (30 min) is shown in Table 4. In ischemia, the level of the full-oxidation metabolite formed by the complete β -oxidation decreased from 20.2% ± 9.2% to 11.7% ± 5.7% and the level of backdiffusion of nonmetabolized BMIPP increased from 27.9% ± 7.7% to 42.3% ± 8.1%. These changes achieved statistical significance (*P* < 0.05).

Metabolism of BMIPP in Tissue

From the TLC profiles of the standard samples, reference (*R_f*) values of triglyceride, BMIPP, α -oxidation metabolite, and full-oxidation metabolite were determined to 0.68, 0.35, 0.30, and 0.20, respectively. On the basis of these values, the fractions from *R_f* = 0.60–0.75 were defined as triglyceride, the fractions from *R_f* = 0.3–0.4 as BMIPP and α -oxidation metabolite (long-chain fatty acids) and the fractions from *R_f* = 0.2–0.3 as short-chain fatty acids (such as full-oxidation metabolite). And then, the fractions from *R_f* 0 to *R_f* 0.2 were regarded as polar lipids.

The metabolites in tissue were calculated from these data, and the percentage of each metabolite group is shown in Table 5. The majority of radioactivity was stored in a triglyceride pool, even in the early phase of both normal and ischemic tissue. However, the value of triglyceride fractions

at 30 min after BMIPP administration significantly decreased from 84.4% ± 4.7% to 76.7% ± 7.8% (*P* < 0.05) with ischemia. This might be due to decreased adenosine triphosphate (ATP) with ischemia. BMIPP and α -oxidized metabolite significantly decreased in the early phase with ischemia (25.9% ± 8.6% to 14.5% ± 2.1%, *P* < 0.01, *t* = 1 min after BMIPP administration; 8.9% ± 5.0% to 4.5% ± 1.7%, *P* < 0.05, *t* = 2 min). This result was compatible with increased backdiffusion of BMIPP from the myocardium in the early phase with ischemia.

Figure 3 shows the comparison of metabolites in tissue and efflux between normal control and ischemia. This figure indicates that the reason why early washout (8 min) of radioactivity increased with ischemia was mainly due to increased backdiffusion of nonmetabolized BMIPP in efflux followed by decreased BMIPP (long-chain fatty acids) in tissue.

DISCUSSION

The results of this study demonstrate that the majority of BMIPP was stored in a triglyceride pool even in the early phase, either in normal or in ischemic tissue, and that, backdiffusion of nonmetabolized BMIPP increased from the myocardium, and BMIPP itself decreased in tissue with ischemia. These results suggest that backdiffusion of nonmetabolized BMIPP might play an important role in perfusion-metabolism mismatch on SPECT images in patients with ischemic heart disease.

The apparent unique, yet not well-understood property of BMIPP, is the mismatch often observed on SPECT images between regional distribution of BMIPP and flow tracers such as ²⁰¹Tl-Cl in patients with ischemic heart disease (5,6)

TABLE 2
Changes in Lactate Consumption and Oxygen Saturation with Ischemia

	Baseline	Occlusion	Perfusion	Final
Lactate consumption (%)	33.5 ± 10.1	-16.5 ± 40.8*	16.3 ± 26.2	34.6 ± 16.7
Oxygen saturation (%)	77.0 ± 3.8	82.2 ± 4.5†	75.3 ± 7.2	72.3 ± 8.6

*P < 0.01 compared with baseline.
†P < 0.05 compared with baseline.

or hypertrophic cardiomyopathy (16). Animal studies with BMIPP have evaluated regional myocardial distribution of this tracer in various cardiac disease models such as hypertensive rats (17,18) or cardiomyopathic hamsters (19). The details, however, are still unclear. It has not yet been completely established if perfusion–metabolism mismatch on SPECT images with ¹²³I-BMIPP is even related to the intracellular oxidative metabolism of BMIPP. There are a lot of studies indicating that increased backdiffusion of BMIPP is the cause of the mismatch on SPECT images; however, there are not many studies that have proved it yet. Matsunari et al. (20) demonstrated that the early clearance of radioactivity could be observed from the mismatch areas using dynamic SPECT, and they speculated that this phenomenon was due to backdiffusion of BMIPP. The real mechanism of “mismatch,” however, is still undetermined. The results of this study demonstrate that the increased washout of radioactivity in the early phase with ischemia was mainly due to the increased backdiffusion of nonmetabolized BMIPP in efflux followed by decreased BMIPP itself in tissue, suggesting that backdiffusion of nonmetabolized BMIPP might play an important part in perfusion–metabolism mismatch on SPECT images in patients with ischemic heart disease. In patients with hypertrophic cardiomyopathy, Tanaka et al. (21) reported that CD36, a myocardial long-chain fatty acid transporter, was involved in myocardial uptake of BMIPP. However, there have been some patients with hypertrophic cardiomyopathy whose SPECT images show low uptake and high clearance of BMIPP (22). These results suggest that the abnormal uptake of BMIPP was related not only to membrane disorder but to intracellular metabolic abnormality. Further examinations might be necessary to elucidate the metabolism of BMIPP in patients with hypertrophic cardiomyopathy.

TABLE 3
Extraction, Retention and Percentage Washout

	Normal control	Ischemia
Extraction (30 sec)	74 ± 12 (%)	77 ± 6 (%)
Retention (30 min)	89 ± 12 (%)	88 ± 6 (%)
Percentage washout (8 min)	50 ± 12 (%)	65 ± 7 (%)*

*P < 0.05 versus normal control.

Clinical Implication

In human studies, many reports discussed the significance of “mismatch” on SPECT images with ¹²³I-BMIPP. Franken et al. (5) reported that segments with more reduced BMIPP uptake than ^{99m}Tc-sestamibi, that is, the mismatch segments, can correspond to either stunned or hibernating myocardium in patients with subacute myocardial infarction. They also showed that mismatching of BMIPP and sestamibi uptake can be predictive of long-term functional recovery after acute myocardial infarction more accurately than dobutamine echo cardiography (23). In addition, Hashimoto et al. (24) indicated that the assessment of the degree and improvement of perfusion–metabolism mismatch by ²⁰¹Tl and ¹²³I-BMIPP SPECT at an acute stage of myocardial infarction might contribute to identifying a future recovery of postischemic myocardial dysfunction. Tamaki et al. (25) suggested that decreased BMIPP uptake relative to ²⁰¹Tl might be a valuable predictor of future cardiac events in patients with myocardial infarction. In animal studies, Fujibayashi et al. (26) demonstrated that the ATP content and the BMIPP accumulation showed a strong positive correlation in a murine model with the intervention of dinitrophenol. Also, Nohara et al. (27) indicated that the myocardial accumulation of BMIPP in the viable ischemic region showed a strong correlation with the ATP content using a canine model with ischemia. These studies indicated ¹²³I-BMIPP could be useful for predicting myocardial viability, functional recovery and risk stratification. The results from this study indicate that BMIPP was well retained in the triglyceride pool of either normal or ischemic canine myocardium; however, the percentage washout in the early phase (8 min)

TABLE 4
Percentage Washout Values of Radioactive Metabolites from Myocardium

	Normal control (%)	Ischemia (%)
Full-oxidation metabolite	20.2 ± 9.2	11.7 ± 5.7*
Intermediate metabolites	25.7 ± 9.9	27.3 ± 9.5
Alpha-oxidation metabolite	26.2 ± 4.3	18.7 ± 2.5†
Back diffusion of BMIPP	27.9 ± 7.7	42.3 ± 8.1†

*P < 0.05 compared with normal control.
†P < 0.01 compared with normal control.

TABLE 5
Percentage Values of Radioactive Metabolites in Tissue

	1 min	2 min	5 min	10 min	15 min	30 min
Normal control						
Triglyceride (%)	42.3 ± 9.6	56.3 ± 6.8	70.0 ± 7.5	75.8 ± 8.4	81.9 ± 5.7	84.4 ± 4.7
Long-chain fatty acids (%)	25.9 ± 8.6	8.9 ± 5.0	4.0 ± 2.3	3.0 ± 1.4	2.0 ± 0.8	2.5 ± 1.0
Short-chain fatty acids (%)	3.9 ± 1.7	6.7 ± 4.0	9.1 ± 5.2	8.5 ± 5.6	4.1 ± 1.9	2.7 ± 1.9
Polar lipids (%)	28.2 ± 7.9	32.6 ± 8.2	22.0 ± 3.4	17.6 ± 6.6	12.3 ± 4.0	9.3 ± 4.9
Ischemia						
Triglyceride (%)	49.2 ± 13.7	60.9 ± 15.2	63.2 ± 8.9	70.3 ± 12.0	74.7 ± 7.8	76.7 ± 7.8*
Long-chain fatty acids (%)	14.5 ± 2.1†	4.5 ± 1.7*	2.7 ± 0.7	3.0 ± 1.0	3.4 ± 2.3	3.0 ± 1.3
Short-chain fatty acids (%)	2.2 ± 1.0*	2.4 ± 1.0*	4.9 ± 1.9	6.4 ± 2.9	4.9 ± 1.8	3.4 ± 1.4
Polar lipids (%)	32.1 ± 14.4	30.5 ± 16.1	29.7 ± 9.2*	22.9 ± 12.5*	18.1 ± 7.8	13.5 ± 5.7

**P* < 0.05 compared with normal control.

†*P* < 0.01 compared with normal control.

increased with ischemia. Considering the difference in heart rate between dogs and humans, one can estimate that an 8-min period after BMIPP administration in a canine model corresponds to 15–20 min in humans (28), which corresponds to the usual period for the initiation of early SPECT imaging. Thus, one can safely say that increased washout from ischemic myocardium in the early phase may produce the mismatch on SPECT images. In addition, the increased washout from ischemic myocardium was mainly due to the increased backdiffusion of nonmetabolized BMIPP followed by decreased β-oxidation. Thus, backdiffusion of nonmetabolized BMIPP may contribute to mismatch on SPECT images in patients with ischemic heart disease.

Study Limitations

Although backdiffusion of nonmetabolized BMIPP contributed to perfusion–metabolism mismatch on SPECT images in patients with ischemic heart disease, a limitation that must be acknowledged is that the efflux protocol and the biopsy protocol were performed separately. Both protocols

must be performed punctually, and bleeding and edema by tissue biopsy were inevitable. The precise procedure of both protocols at the same time might be impossible without contamination to each other.

Also, in the biopsy protocol of this study, wall thickening and metabolic parameters were not measured because of the muscle damage by biopsies being performed six times, inevitable bleeding by tissue samplings and necessity of hemostasis after biopsy, although hemodynamics did not change significantly during the protocol.

In the metabolic analysis using TLC, BMIPP and α-oxidation metabolite could not be separated from each other. This was the same result as previously reported (15). The separation of BMIPP from α-oxidation metabolite needs HPLC analysis, which requires 4 to 5 dogs killed at each timing after the administration of BMIPP, that is, at least 50 dogs are required in the biopsy protocols. This would be undesirable for the protection of animals. In addition, this study was enough to show that backdiffusion of BMIPP might play an important role of “mismatch” on SPECT

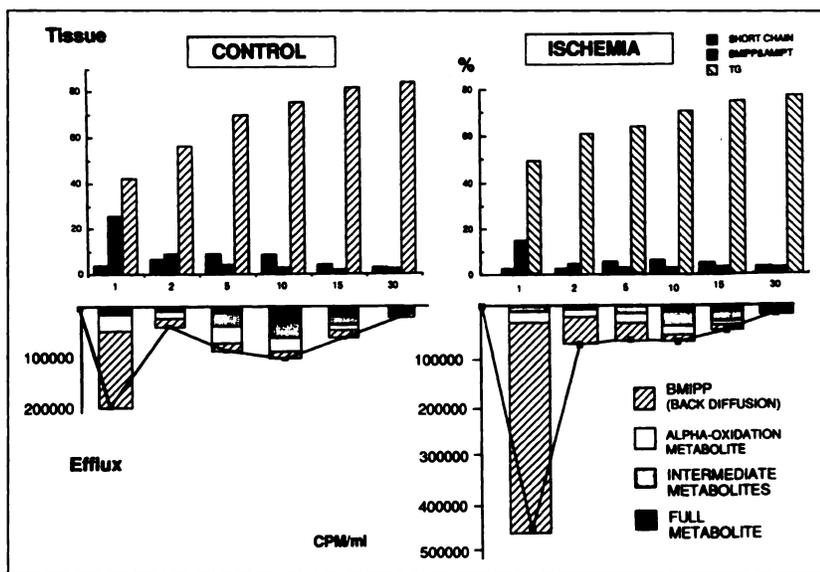


FIGURE 3. Mean washout of each metabolite from the myocardium (in efflux) and in myocardial tissue. Comparisons between normal control and ischemia. SHORt CHAIN = short-chain fatty acids catabolized from BMIPP, such as full-oxidation metabolite; AMIPT = α-oxidation metabolite; TG = triglyceride.

images because of the combined studies of biopsy with efflux.

CONCLUSION

Backdiffusion of nonmetabolized BMIPP increased and full-oxidation metabolite formed by complete β -oxidation decreased from the myocardium, and BMIPP (long-chain fatty acids) decreased in tissue with ischemia. Thus, increased backdiffusion of nonmetabolized BMIPP might play an important role in perfusion-metabolism mismatch on SPECT images in patients with ischemic heart disease.

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REFERENCES

1. Knapp FF Jr, Kropp J. Iodine-123-labeled fatty acids for myocardial SPECT: current status and future perspectives. *Eur J Nucl Med.* 1995;22:361-381.
2. Knapp FF Jr, Kropp J, Goodman MM, et al. The development of iodine-123-methyl-branched fatty acids and their applications in nuclear cardiology. *Ann Nucl Med.* 1993;7:1-14.
3. Knapp FF Jr, Goodman MM, Ambrose KR, et al. The development of radioiodinated 3-methyl-branched fatty acids for evaluation of myocardial disease by single photon techniques. In: van der Wall EE, ed. *Noninvasive Imaging of Cardiac Metabolism.* Dordrecht, The Netherlands: Martius Nijhoff Publishers; 1987:159-201.
4. Fujibayashi Y, Yonekura Y, Kawai K, et al. Basic studies on I-123- β -methyl- p -iodophenylpentadecanoic acid (BMIPP) for myocardial functional diagnosis: effect of beta-oxidation inhibitor. *Jpn J Nucl Med.* 1988;25:1131-1135.
5. Franken PR, De Geeter F, Dendale P, et al. Abnormal free fatty acid uptake in subacute myocardial infarction after coronary thrombolysis: correlation with wall motion and inotropic reserve. *J Nucl Med.* 1994;35:1758-1765.
6. Tamaki N, Kawamoto M, Yonekura Y, et al. Regional metabolic abnormality in relation to perfusion and wall motion in patients with myocardial infarction: assessment with emission tomography using iodinated branched fatty acid analog. *J Nucl Med.* 1992;33:659-667.
7. Kawamoto M, Tamaki N, Yonekura Y, et al. Combined study with I-123 fatty acid and thallium-201 to assess ischemic myocardium: comparison with thallium redistribution and glucose metabolism. *Ann Nucl Med.* 1994;8:847-854.
8. Takeishi Y, Sukekawa H, Saito H, et al. Impaired myocardial fatty acid metabolism detected by ^{123}I -BMIPP in patients with unstable angina pectoris: comparison with perfusion imaging by $^{99\text{m}}\text{Tc}$ -sestamibi. *Ann Nucl Med.* 1995;9:125-130.
9. Hosokawa R, Nohara R, Fujibayashi Y, et al. Metabolic fate of iodine-123 BMIPP in canine myocardium after administration of etomoxir. *J Nucl Med.* 1996;37:1836-1840.
10. Hosokawa R, Nohara R, Fujibayashi Y, et al. Myocardial kinetics of iodine-123-BMIPP in canine myocardium after regional ischemia and reperfusion: implications for clinical SPECT. *J Nucl Med.* 1997;38:1857-1863.
11. Hale SL, Alker KJ, Kloner RA. Evaluation of non-radioactive, colored microspheres for measurement of regional myocardial blood flow in dogs. *Circulation.* 1988;78:428-434.
12. Hartley CJ, Laston LA, Michael LH, et al. Doppler measurement of myocardial thickening with a single epicardial transducer. *Am J Physiol.* 1983;245:H1066-H1072.
13. Fujibayashi Y, Nohara R, Hosokawa R, et al. Metabolism and kinetics of iodine-123-BMIPP in canine myocardium. *J Nucl Med.* 1996;37:757-761.
14. Folch J, Lees M. Proteolipides, a new type of tissue lipoproteins. *J Biol Chem.* 1951;191:807-817.
15. Yamamichi Y, Kusuoka H, Morishita K, et al. Metabolism of ^{123}I -BMIPP in perfused rat hearts. *J Nucl Med.* 1995;36:1043-1050.
16. Kurata C, Tawarahara K, Taguchi T, et al. Myocardial emission computed tomography with ^{123}I -labeled beta-methyl-branched fatty acid in patients with hypertrophic cardiomyopathy. *J Nucl Med.* 1992;33:6-13.
17. Yonekura Y, Brill AM, Som P, et al. Quantitative autoradiographic measurement of regional myocardial substrate utilization in hypertensive rats. *Science.* 1985;227:1494-1496.
18. Yamamoto K, Som P, Brill AB, et al. Dual tracer autoradiographic study of β -methyl (1- ^{14}C) heptadecanoic acid and 15- p -(^{131}I)-iodophenyl- β -methylpentadecanoic acid in normotensive and hypertensive rats. *J Nucl Med.* 1986;27:1178-1183.
19. Kurata C, Kobayashi A, Yamazaki N. Dual tracer autoradiographic study with ^{201}Tl and radioiodinated fatty acid in cardiomyopathic hamsters. *J Nucl Med.* 1989;30:80-87.
20. Matsunari I, Saga T, Taki J, et al. Kinetics of iodine-123-BMIPP in patients with prior myocardial infarction: assessment with dynamic rest and stress images. Compared with stress thallium-201 SPECT. *J Nucl Med.* 1994;35:1279-1285.
21. Tanaka T, Sohmiya K, Kawamura K, et al. Is CD 36 deficiency an etiology of heredity hypertrophic cardiomyopathy? *J Mol Cell Cardiol.* 1997;29:121-127.
22. Takeishi Y, Chiba J, Abe S, et al. Heterogeneous myocardial distribution of iodine-123 15-(p -iodophenyl)-3- R,S -methylpentadecanoic acid (BMIPP) in patients with hypertrophic cardiomyopathy. *Eur J Nucl Med.* 1992;19:775-782.
23. Franken PR, Dendale P, Pegeeter F, et al. Prediction of functional outcome after myocardial infarction using BMIPP and sestamibi scintigraphy. *J Nucl Med.* 1996;37:718-722.
24. Hashimoto A, Nakata T, Tsuchihashi K, et al. Postischemic functional recovery and BMIPP uptake after primary percutaneous transluminal coronary angioplasty in acute myocardial infarction. *Am J Cardiol.* 1996;77:25-30.
25. Tamaki N, Tadamura E, Kudoh T, et al. Prognostic value of iodine-123-BMIPP fatty acid analog imaging in patients with myocardial infarction. *Eur J Nucl Med.* 1996;23:272-279.
26. Fujibayashi Y, Yonekura Y, Takemura Y, et al. Myocardial accumulation of iodinated beta-methyl-branched fatty acid analog, iodine-125-15-(p -iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP), in relation to ATP concentration. *J Nucl Med.* 1990;31:1818-1822.
27. Nohara R, Okuda K, Hosokawa R, et al. Evaluation of myocardial viability with iodine-123-BMIPP in a canine model. *J Nucl Med.* 1996;37:1403-1407.
28. Hosokawa R, Nohara R, Hirai T, et al. Why dobutamine increased the uptake of free fatty acids in stunned myocardium? Experimental investigation using I-123 BMIPP [abstract]. *J Nucl Med.* 1998;39(suppl):158P.