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Comparison of Fatty Acid Tracers FTHA and BMIPP During Myocardial Ischemia and Hypoxia

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To study the sensitivity of two fatty acid tracers to changes in beta-oxidation, the myocardial retention kinetics of ^{125}I-iodine-15-(p-iodophenyl)-3(R,S)-methylpentadecanoic acid (BMIPP) and 14-^{18}F-fluoro-6-thia-heptadecanoic acid (FTHA) were compared in states of oxygen deprivation due to ischemia and hypoxia. **Methods:** Nineteen swine were studied by extracorporeal perfusion of the three coronary arteries. Fatty acid beta-oxidation rates were determined by infusion of tritiated palmitate into the left anterior descending artery (LAD) and by measurement of labeled water production in the LAD perfusion bed. After a baseline period of 30 min, animals were divided into three groups and subjected to a 50-min intervention period. For the control group, there was no change in perfusion; for the ischemia group, there was a 60% decrease in LAD perfusion; and for the hypoxia group, the perfusion rate was unchanged, but venous blood was used as the LAD perfusate. Continuous infusion of FTHA and BMIPP into the LAD started 10 min into the intervention period and continued until the end of the intervention period. Retention rates of the two tracers were compared between the LAD and circumflex perfusion beds. **Results:** No difference in beta-oxidation rate occurred from the baseline to the intervention period in the control group. A 50% reduction in beta-oxidation occurred in the ischemia group, and an 80% reduction occurred in the hypoxia group. No difference in retention of BMIPP or FTHA occurred in the control group. In the ischemia group, reduction in retention of both tracers occurred. However, in the hypoxia group, FTHA uptake was unchanged, whereas BMIPP retention increased compared to the circumflex arterial bed. **Conclusion:** Decreased retention of both BMIPP and FTHA occurred with ischemia, despite the known differences in metabolism of the two tracers. This difference in metabolism was further highlighted in the setting of hypoxia with increased BMIPP uptake. Thus, these results suggest that uptake of both FTHA and BMIPP tracks reduction of fatty acid utilization in myocardial ischemia but fails in tracking reduction of fatty acid oxidation during hypoxia.

Key Words: fatty acid tracer; FTHA; BMIPP; myocardial ischemia; myocardial hypoxia

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Long-chain fatty acids are the main energy source for the heart and are rapidly metabolized by beta-oxidation under normal conditions. However, in settings of reduced oxygen delivery such as ischemia and hypoxia, it is well known that fatty acid oxidation is vastly decreased and accompanied by a decline in mechanical function. To noninvasively assess changes in fatty acid metabolism, several fatty acid tracers have been developed over the last decade for imaging with standard gamma cameras or PET. Among the widely investigated tracers has been the 15-carbon methyl-branched fatty acid analog ^{125}I-iodine-15-(p-iodophenyl)-3(R,S)-methylpentadecanoic acid (BMIPP). Most of the tracer is rapidly incorporated into triacylglycerols in the cytosol, but a portion undergoes alpha-oxidation (*1–3*). BMIPP has also been shown in cultured cell (*4*) and isolated organ (*5*) preparations to be insensitive to changes in beta-oxidation. Extensive clinical trials, however, have reported a mismatch between BMIPP uptake and blood flow distribution (*6–8*).

Recently, a sulfur-substituted fatty acid tracer analog 14-^{18}F-

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fluoro-6-thia-heptadecanoic acid (FTHA) has been introduced (*9*). This analog is a ^{17}C fatty acid with a sulfur heteroatom that blocks beta-oxidation during the second cycle of beta-oxidation. FTHA, in contrast to BMIPP, has been shown to be sensitive to changes in fatty acid beta-oxidation induced by inhibitors (*10*) or lactate (*11*). A strong correlation has also been shown between FTHA uptake rate and rate–pressure product (*12*). The goal of this study was, therefore, to correlate the retention of BMIPP and FTHA to labeled water production from tritiated palmitate in the settings of depressed beta-oxidation by ischemia and hypoxia using the extracorporeally perfused swine heart preparation. The central hypothesis was that intermediary metabolism of BMIPP would reduce the correlation of retention with beta-oxidation rates in comparison to FTHA.

MATERIALS AND METHODS

Synthesis of Substrate

FTHA was synthesized according to the method described by DeGrado (*9*). The precursor benzyl-14(R,S)-tosyloxy-6-thia-heptadecanoic acid was labeled with ^{18}F by nucleophilic substitution under standard conditions to give ^{18}F-FTHA. The ^{18}F-fluoride was produced from bombardment of a ^{18}O target with 11-MeV protons in the Department of Medical Physics RDS Cyclotron (CTI Inc., Knoxville, TN). Fluorine-18-FTHA was then purified by high-performance liquid chromatography according to published methods (*9*). BMIPP was obtained commercially (MARA Inc., Marcus Hook, PA), and the compound was labeled with ^{125}I-NaI according to DeGrado et al. (*5*).

Surgical Procedure

The surgical preparation and the anesthesia were approved by the University of Wisconsin-Madison Research Animal Resources Committee. The extracorporeally perfused heart model that was used in this study has been described in detail elsewhere (*13,14*). Briefly, swine weighing 45–50 kg were preanesthetized with ketamine (11 mg/kg), atropine (0.8 mg) and acepromazine (1.1 mg/kg) intramuscularly. Anesthesia was initially introduced by adding sodium thiopental (1.1 mg/kg intravenously) and maintained with alpha-chloralose (1.5 g initially, followed by 0.5 g/hr) and a subcutaneous injection of morphine sulfate (45 mg/hr). Tracheostomy was performed in the supine position, and the animal was ventilated through an endotracheal tube using 100% oxygen. The sternum was removed, and the right internal carotid artery and the internal jugular vein were isolated. A double-tipped pressure transducer (Millar) was inserted to measure aortic and left ventricular pressure (LVP) and its first derivative (dP/dt). The femoral artery and vein were cannulated. The right coronary artery (RCA), left main artery and the left anterior descending coronary arteries (LADs) were also cannulated and perfused by three separate perfusion pumps. The LAD was ligated proximally during cannulation so that all left main perfusion was directed down the circumflex (CIRC) arterial bed. Blood was withdrawn from the cannulated femoral artery and returned to each coronary artery by three perfusion pumps. At the time of cannulation, an initial bolus of 20,000 units of heparin was given, followed hourly by 10,000-unit boluses. The CIRC and the LAD perfusion circuits contained a pump for arterial sampling (7 ml/min) to prevent variations in the arterial flow during sampling. For the control and ischemia groups (see below), a mixing chamber (100 ml) for infusion of microspheres, cardiogreen, FTHA and BMIPP into the arterial perfusate was placed in the arterial line after the RCA pump but before the CIRC and LAD pumps. For the hypoxia group, the mixing chamber was omitted, and ports were placed in the CIRC and LAD arterial lines for the infusion of separate microspheres, cardiogreen, FTHA and BMIPP. A separate port was present before the LAD pump for infusion of tritiated palmitate for all three groups. Furthermore, the CIRC and LAD veins were cannulated to obtain venous samples with the CIRC cannulation performed as high as possible to ensure more CIRC retrieval. The blood from these two veins was drained into the chest cavity and returned through a reservoir to the femoral vein. Heart rate was measured using electrocardiograph leads, and mechanical performance was obtained using thickness crystals placed in the LAD perfusion bed.

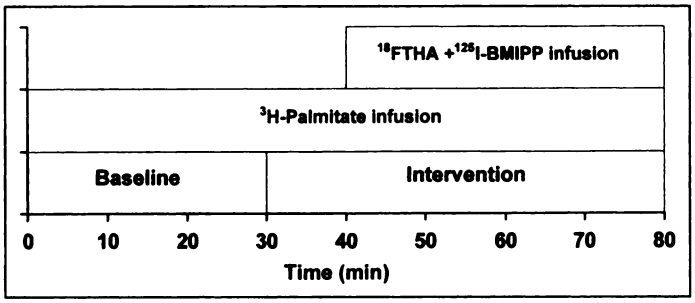

FIGURE 1. Protocol timeline shows time intervals for baseline (0–30 min) and intervention (30–80 min). Infusion of ^{3}H-palmitate occurred throughout study period, and labeled FTHA and BMIPP were infused from time point 40 min and continued until end of study.

Experimental Protocol

Three groups of animals were studied. These groups included the control group (n = 5), the ischemia group (n = 9) and the hypoxia group (n = 5). Each animal underwent a control period for the first 30 min of the study period, followed by intervention for the next 50 min, as shown in Figure 1. In the control group, perfusion flows were kept constant throughout the study, whereas in the ischemia group, ischemia was precipitated by reducing the LAD flow by 60%. Hypoxia was induced by perfusing the LAD bed with venous blood from a right atrial catheter. Mechanical data were obtained every 10 min throughout the study. Arterial and venous samples were obtained every 10 min throughout the experiment to measure blood gases for determination of myocardial oxygen consumption (MVO_2). In addition, arterial and venous blood samples were obtained at 20, 40, 60 and 80 min of perfusion for measuring lactate levels (1500 YSI Sport lactate analyzer; Yellow Springs Instrument Co., Yellow Springs, OH). Cardiogreen was infused into the mixing chamber (or directly into the LAD and CIRC arterial lines for the hypoxia group) for 5 min at selected intervals to obtain the dilution factor from the arterial and venous samples of unlabeled blood entering the respective bed. Cardiogreen concentration was measured by a spectrophotometer and the dilution factor K was then used in the calculations.

Radioactive 9,10-^{3}H-palmitate (100–150 μCi per animal) was infused into the LAD starting at time 0 min and continuing throughout the experiment. Arterial and venous LAD blood samples for measuring ^{3}H-H_2O were collected every 10 min throughout the study. Infusion of ^{18}FTHA (4.6 ± 0.5 mCi per animal) and ^{125}I-BMIPP (24 ± 9 μCi per animal) in a volume of 50 ml was started at 40 min and continued until 80 min. The LAD and CIRC arterial and venous blood samples were obtained at time points 40, 50 and 60 min and every 5 min thereafter. In addition, the femoral blood samples for ^{18}F-FTHA and ^{125}I-BMIPP were obtained at time points 60, 70 and 80 min.

Radioactive microspheres (5 μCi) were introduced during baseline and intervention. In all groups, the withdrawal rate for the arterial reference sample was 1.94 ml/min over a 6-min period.

At the end of the experiment, both the CIRC and the LAD perfusion beds were stained simultaneously with the right coronary bed perfused to give balanced pressure during the staining. The perfusion beds were dissected free and cut into epicardial and

TABLE 1
Mean Myocardial Blood Flow Results by Microsphere Technique

Perfusion data	Control group	Ischemia group	Hypoxia group
LAD baseline	1.9 ± 0.6	1.4 ± 0.5	1.3 ± 0.3
LAD intervention	2.1 ± 1.1	0.6 ± 0.2*†	2.3 ± 0.9
CIRC baseline	1.9 ± 0.7	1.6 ± 0.7	1.4 ± 0.8
CIRC intervention	2.0 ± 1.3	1.8 ± 1.1	1.4 ± 0.4
LAD-to-CIRC ratio			
Baseline	1.0 ± 0.1	0.9 ± 0.2	1.4 ± 1.1
Intervention	1.1 ± 0.2	0.4 ± 0.1‡§	2.0 ± 1.5

*$p < 0.05$ vs. ischemia group baseline.
†$p < 0.005$ vs. control group intervention.
‡$p < 0.00001$ vs. ischemia group baseline.
§$p < 0.00001$ vs. control group intervention.
LAD = left anterior descending artery; CIRC = circumflex.
p values for significant comparisons are shown. Values are mean ± s.d.

endocardial sections from both perfusion beds and immediately counted for ^{18}F-FTHA and ^{125}I-BMIPP followed by a delayed counting of the microspheres.

Biochemical and Tissue Analysis

Arterial and venous blood samples were centrifuged at 1500 × *g* for 10 min to obtain plasma. This plasma was then analyzed for ^{3}H-H_2O as described by Saddik and Lopaschuk (*15*). Beta-oxidation rate (R_{FFA}, in $\mu mol \cdot hr^{-1} \cdot g$ dry weight^{-1}) from tritiated palmitate was calculated according to the formula:

$$R_{FFA} = \frac{(\Delta^3 H\text{-}H_2O \cdot Q_{LAD} \cdot 60)}{(K \cdot ASA_{FFA} \cdot LAD_{dry\ wt})},$$

where Δ^3H-H_2O is the difference between arterial and venous tritiated water levels (dpm/ml), Q_{LAD} is the arterial LAD blood flow (ml/min), K is the dilution factor obtained from using cardiogreen, ASA_{FFA} is the arterial specific activity of radioactive palmitate (dpm/μmol) and $LAD_{dry\ wt}$ is the LAD perfusion bed weight expressed as its dry weight (*11*). Arterial plasma fatty acid concentration was obtained by colorimetric measurements using a fatty acid analysis kit (NEFA-C; Wako Chemicals, Richmond, VA).

Because the LAD and CIRC perfusion beds did not have a common arterial input in the hypoxia group, the uptake data for all groups were normalized by dividing the tissue radioactivity concentrations (counting rate/g) in each bed at the end of the perfusion by the time integrals of their respective measured input functions (counting rate/ml blood). Tissue retentions for both ^{18}F-FTHA and ^{125}I-BMIPP were expressed as the ratios of the normalized radioactivity concentrations in the LAD bed to those in the CIRC bed.

Calculation of blood flow based on microsphere activity was performed using the arterial reference method (*16*).

Statistics

All data are given as mean ± s.d. if not otherwise indicated. Baseline values for the hemodynamic parameters, including free fatty acid and lactate levels, were averaged for time points 0–30 min and compared to the intervention values (time points 40–80 min). For tritiated water production, the baseline values included time points 10–30 min and were compared to the intervention interval, which included time points 40–80 min. Fluorine-18 and ^{125}I retention ratios were compared to those of the control group using unpaired two-tailed Student's t-test. Paired two-tailed Student's t-tests were used to compare hemodynamic parameters and fatty acid and lactate levels for the three groups and of the retention ratios uptake of FTHA and BMIPP in the hypoxia group. Two-tailed Student's t-test, paired or unpaired, was also used to test microsphere blood data. Specific comparisons performed were intervention versus baseline values for both perfusion beds for all three groups and ischemia and hypoxia intervention versus control intervention. The ratio of LAD-to-CIRC perfusion for the three groups was performed in a similar fashion. Bonferroni correction was performed for the multiple comparisons. p values that were <0.05 were considered significant.

RESULTS

Myocardial blood flow by radioactive microspheres showed the expected decline in LAD perfusion for the ischemia group during ischemia (0.6 ± 0.2 ml/min/g) compared to baseline (1.4 ± 0.5 ml/min/g, $p < 0.005$). Otherwise, no significant changes were seen in LAD perfusion for the control or the hypoxia group nor were there any changes in the CIRC perfusion for any of the three groups for either intervention (Table 1). Similarly, the ratio of LAD-to-CIRC blood flow decreased in the intervention period for the ischemia group (0.4 ± 0.1), with no change in the control or hypoxia groups (Table 1).

The hemodynamic and metabolic parameters are shown in Table 2. In the control group, no statistical changes were seen between baseline and the last 50 min of intervention period for any of the measurements. However, dP/dt declined during

TABLE 2
Hemodynamic and Metabolic Parameters for the Three Study Groups

Group	LVP (mmHg)	dP/dt (mmHg/sec)	HR (bpm)	%SS (%)	MVO_2 (ml/g/min)	FFA (mM)	Lactate (A-V) mM
Control							
Baseline	73 ± 17	1793 ± 724	136 ± 22	104 ± 34	7.3 ± 2.1	0.48 ± 0.06	0.34 ± 0.19
Intervention	74 ± 17	173 ± 744	129 ± 26	136 ± 56	6.8 ± 2.3	0.44 ± 0.15	0.17 ± 0.34
p value	NS	NS	NS	NS	NS	NS	NS
Ischemia							
Baseline	75 ± 12	1536 ± 400	128 ± 39	100 ± 33	7.3 ± 1.8	0.45 ± 0.08	0.52 ± 0.58
Intervention	71 ± 16	911 ± 390	125 ± 38	23 ± 46	3.6 ± 1.1	0.42 ± 0.15	−0.70 ± 0.42
p value	NS	0.0006	NS	0.006	0.00002	NS	0.001
Hypoxia							
Baseline	66 ± 7	1361 ± 204	150 ± 22	76 ± 10	8.7 ± 2.9	0.52 ± 0.30	0.46 ± 0.14
Intervention	39 ± 10	709 ± 246	114 ± 36	29 ± 36	1.7 ± 0.7	0.32 ± 0.14	−0.51 ± 0.54
p value	0.017	<0.0001	NS	NS	0.007	NS	0.017

LVP = left ventricular pressure; dP/dt = first derivative of LVP; HR = heart rate; %SS = percentage systolic shortening; MVO_2 = myocardial oxygen consumption; FFA = free fatty acid; NS = not significant.
Values are given as mean ± s.d.

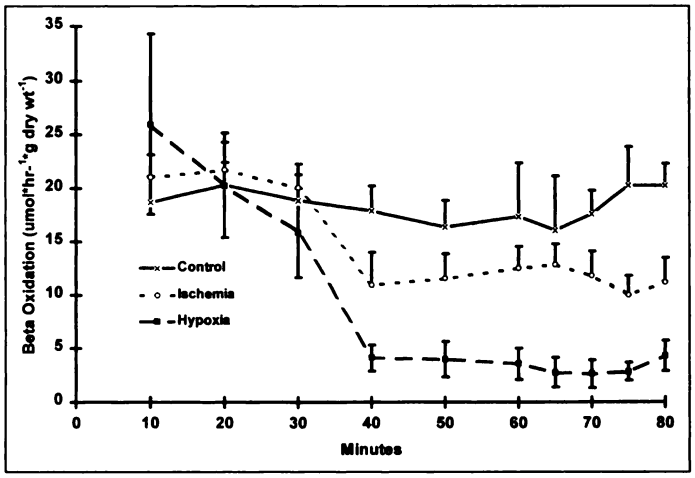

FIGURE 2. Beta-oxidation (μmol·hr^{-1}·g dry weight^{-1}) for three study groups is shown. Beta-oxidation was calculated from left anterior descending artery arteriovenous differences as explained in Materials and Methods section for control group (×), ischemia group (○) and hypoxia group (■). Values are given as mean ± s.e.m.

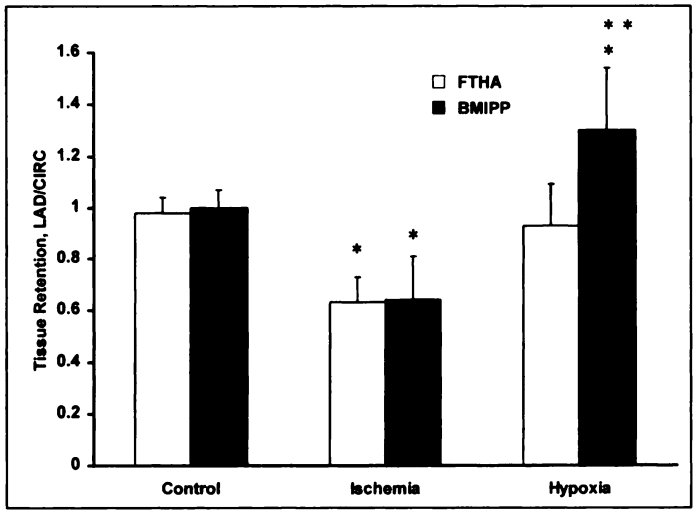

FIGURE 3. Extraction of FTHA (white bars) and BMIPP (black bars) is illustrated as ratio of tissue retention between left anterior descending artery and circumflex perfusion beds for control, ischemia and hypoxia groups. Values are given as mean ± s.d. *$p < 0.05$ for ratio of tracer activity in ischemia or hypoxia versus control groups; **$p < 0.009$ for BMIPP versus FTHA ratio within hypoxia group.

intervention compared to baseline for both the ischemia ($p < 0.0006$) and the hypoxia groups ($p < 0.0001$). In addition, the hypoxia group showed a decline in LVP ($p < 0.017$) during hypoxia that was not noted in the ischemia group. In all groups, the values for percentage systolic shortening (%SS) were normalized to the initial value for each animal to minimize variations between the animals, a procedure that is routinely performed in this laboratory when the extracorporeally perfused animal model is used. As expected, the %SS declined during intervention for the ischemia group ($p < 0.006$ compared to baseline values), but no significant changes were seen during hypoxia. The expected decrease in MVO_2 was seen during ischemia ($p < 0.00002$) with the expected decline during hypoxia ($p < 0.007$). Furthermore, a significant change in arteriovenous difference of lactate was noticed in both intervention groups compared to baseline values ($p < 0.0014$ for the ischemia group and $p < 0.017$ for the hypoxia group).

Fatty acid oxidation rates were determined by tritiated water production from labeled palmitate and are shown in Figure 2. A decrease compared to baseline values was seen in both intervention groups. In the ischemia group, beta-oxidation decreased from 21 ± 11 to 11 ± 6 μmol·hr^{-1}·g dry weight^{-1}, (mean ± s.d.; $p < 0.04$), with an even greater decline in beta-oxidation during hypoxia compared to baseline values (21 ± 13 versus 3.5 ± 2.5 μmol·hr^{-1}·g dry weight^{-1}; $p < 0.02$).

The tracer uptake rates for FTHA and BMIPP were calculated as described in the Materials and Methods section. In Figure 3, the results are given as a ratio between the normalized radioactivity in the LAD and CIRC perfusion beds for each group. For the control group, the ratio for both the tracers was unity. In the ischemia group, a reduction relative to the control group was seen in the LAD-to-CIRC perfusion bed ratio for both FTHA (0.6 ± 0.1; $p < 0.00001$) and BMIPP (0.6 ± 0.2; $p < 0.0008$). On the other hand, the hypoxia group showed no significant change in the ratio for FTHA; however, the BMIPP retention ratio increased 30% compared to the BMIPP ratio for the control group (1.3 ± 0.2 versus 1.0 ± 0.2, $p < 0.028$) and by 44% compared to the FTHA ratio for the hypoxia group (0.9 ± 0.2, $p < 0.009$).

In summary, both the ischemic and the hypoxic interventions caused reduced oxygen supply for myocardial needs, which resulted in mechanical dysfunction, reduced beta-oxidation and net lactate production. In the ischemia group, the retention of FTHA and BMIPP was equally reduced in the LAD perfusion bed. In the hypoxia group, however, a net increase in the retention of BMIPP occurred despite reduced beta-oxidation, whereas FTHA retention was unchanged. These results suggest that both FTHA and BMIPP retention are not solely related to beta-oxidation and that other mechanisms are involved. The increase in BMIPP retention during hypoxia is consistent with the tracer being more sensitive to intermediary fatty acid metabolism than FTHA.

DISCUSSION

There is significant interest in the development of a noninvasive technique for the quantitative assessment of the beta-oxidation rate of fatty acids under a broad range of pathological conditions. The ^{18}F-labeled thia-substituted fatty acid analog FTHA was developed to specifically target the beta-oxidation pathway (*9*). BMIPP is another metabolically trapped fatty acid tracer that has received a great deal of attention as a SPECT tracer of fatty acid metabolism, due to its favorable imaging characteristics and dependence on intracellular metabolic events. Therefore, it was our goal to directly compare the myocardial retention of these two metabolically trapped tracers in response to myocardial ischemia and hypoxia, two conditions known to cause suppression of fatty acid oxidation in the heart. Animals were divided into three groups with each group undergoing a baseline period of 30 min, followed by a 50-min intervention period of no change in perfusion, reduced perfusion or reduced oxygen tension. Tritiated palmitate was infused during the total experimental study period, whereas ^{18}F-FTHA and ^{125}I-BMIPP were coinfused during the last 40 min of the intervention period. Tissue samples were obtained at the end of the experimental period to determine the tissue retention of ^{18}F and ^{125}I in both the LAD and CIRC beds.

No differences in hemodynamic or metabolic parameters were seen in the control group, and as expected, there was no difference in retention of FTHA or BMIPP between the two perfusion beds. In the ischemia group, the reduction in blood flow resulted in a significant reduction in LVP, dP/dt, %SS and myocardial oxygen consumption, consistent with an ischemic response. Net lactate production also occurred with the shift in metabolism to anaerobic glycolysis. Tritiated water production decreased by 50%, indicative of the suppression of beta-

oxidation during ischemia. Net myocardial retention of both FTHA and BMIPP was less than that in the aerobically perfused CIRC bed. The magnitude of these reductions was the same as the reduction in beta-oxidation rate from baseline to ischemia. Thus, both tracers exhibited a decline in retention, paralleling the reduction in fatty acid oxidation.

Both BMIPP and FTHA are retained tracers, developed for the assessment of fatty acid metabolism using standard gamma camera imaging and PET, respectively. BMIPP is a ^{15}C fatty acid modified with a methyl group to prevent further metabolism. Although originally designed to track beta-oxidation, BMIPP has been shown to be metabolized predominantly in the cytosol, where it is mainly converted to triacylglycerols, and, to a lesser degree, to undergo alpha-oxidation, with the resulting product further metabolized by beta-oxidation (*2,17*). The conversion of BMIPP to triacylglycerols occurs soon after injection, with 80% converted within 5 min (*2*). Furthermore, in isolated rat heart preparations, BMIPP uptake kinetics have been insensitive to inhibition of fatty acid oxidation by the carnitine-palmitoyl transferase I inhibitor [5-(4-chlorophenyl)-pentyl]oxirane-2-carboxylate (POCA) (*5*).

The recently introduced fatty acid analog FTHA has a ^{17}C backbone with a sulfur heteroatom to block further metabolism. Mouse and swine studies have shown that it is rapidly cleared from the bloodstream and has a high myocardial retention without flow dependency for uptake (*11,12*). Isolated rat heart studies have shown decreased uptake of FTHA during inhibition of beta-oxidation with POCA (*18*). FTHA was also responsive to changes in beta-oxidation introduced by a shift in substrate utilization from fatty acids to lactate, obtained by lactate infusion (*11*). As opposed to BMIPP, only 6% of FTHA is converted to triacylglycerols, and over 80% is protein-bound in mice (*10*). Swine experiments have also confirmed the high rate of protein binding (79% ± 10%) (*11*).

Both tracers exhibited decreased retention with ischemia, despite their lack of similarity in metabolic handling in the myocardium. The reduction of delivery of tracer to the ischemic myocardium by virtue of lower perfusion is equal for both tracers. Both tracers must be activated to form their respective acyl-CoA thioesters before further metabolic steps may proceed within the myocytes. Fatty acid activation is reduced in ischemic conditions by decreases in cytosolic levels of the cofactors CoA-serum hepatitis and adenosine triphosphate and by increased feedback inhibition by accumulated long-chain fatty acyl-CoA, adenosine monophosphate and inorganic phosphate (*19–21*). Thus, ischemia may be associated with similar reductions in accumulation of FTHA and BMIPP metabolites by suppression at the activation step. Alternatively, concurrent tracer reduction may reflect similar reductions in both oxidative and lipid incorporation pathways, although it is commonly held that triacylglycerol formation is increased in ischemic conditions (*22*).

In the hypoxia group, the reduction of tissue oxygen caused by a reduction in perfusate oxygenation (rather than flow rate as in ischemia) was associated with similar changes in myocardial oxygen consumption and net lactate production, as in the ischemia group. As opposed to the ischemia group, a decrease in systemic pressure occurred in the hypoxia group, presumably a reflex-mediated response to the deoxygenated perfusate. A decrease in LVP dP/dt also occurred, without a significant change in %SS. Suppression of beta-oxidation was also present but to a greater degree than in the ischemia group (80%). Despite the suppression of beta-oxidation, increased retention of BMIPP and no change in the retention of FTHA were noted.

Under normal conditions, fatty acids are the main energy source of the heart. In settings of decreased myocardial oxygenation, the metabolic pattern changes, and in both ischemia and hypoxia, fatty acid oxidation decreases (*23,24*), with an elevation in free fatty acids and triacylglycerol levels (*25,26*). The increase in amphiphiles has been shown to cause impaired membrane integrity (*27*) and depressed myocardial contractility (*13*). Both ischemia and hypoxia are associated with stimulation of glycolysis (*24,26,28*) in whole-animal preparations, with glycolysis becoming a major metabolic pathway for energy production. Even with the increase in glycolysis, adenosine triphosphate levels are reduced in both conditions. However, despite the decrease in myocardial oxygenation in both conditions, ischemia and hypoxia differ in other metabolic aspects due to the continued washout of hydrogen ions, lactate and other metabolites in the hypoxic state (with maintenance of perfusion rate). Rovetto et al. (*29*) demonstrated, in isolated rat hearts, the suppression of glycolysis in ischemic hearts by the inhibition of glyceraldehyde-3-phosphate dehydrogenase with increasing cellular lactate and hydrogen ion concentrations. In their preparation, glycolytic rates were suppressed in ischemia to 50% of control rate, whereas anoxia was associated with a doubling of glycolytic rate compared to control (*30*).

Two potential mechanisms for the increase in BMIPP retention with hypoxia are the increase in cytosolic esterification of fatty acids and the stimulation of glycolysis. In hypoxia, it has been shown from time-activity curves using a four-component mathematical model (*24*) that the esterification process was significantly higher during anaerobic conditions than during aerobic conditions. Thus, the increase in esterification could explain the increase in BMIPP retention during hypoxia. Alternatively, Yamamichi et al. (*31*) demonstrated the subcellular localization of BMIPP in relation to substrate availability in isolated rat heart preparations. In particular, in the setting of perfusate containing glucose and insulin, net myocardial uptake of BMIPP was increased to a greater extent than with the substitution of the fatty acid oleate, suggesting a relationship of BMIPP uptake with carbohydrate metabolism. Interestingly, in the same experiments, cold BMIPP did not suppress fatty acid oxidation, confirming the results of DeGrado et al. (*5*).

The lack of difference in retention of FTHA in hypoxia was not expected, particularly with the known low rate of triglyceride incorporation (*10,11*). Further experiments are necessary to determine the cellular fate of FTHA during hypoxia because available mouse and swine data obtained in normoxic conditions would predict that FTHA retention would be suppressed with hypoxia. In particular, given the degree of suppression of water production from tritiated palmitate, it would be expected that FTHA retention would also be decreased. It is conceivable that some of the retention may be related to mitochondrial membrane deposition of FTHA, but it is expected with the degree of inhibition of beta-oxidation that the carnitine-palmitoyl transferase activity would also be suppressed. It is also possible that triglyceride incorporation of the thia-fatty acid is significant in the hypoxic condition. Other alternative explanations for residual binding of FTHA in hypoxic myocardium are differential incorporation into phospholipids, differential binding of FTHA by intracellular fatty acid binding proteins and differential feedback inhibition of beta-oxidation in comparison to natural fatty acids. Further radioanalytical studies are necessary to clarify this question.

CONCLUSION

Net retention of FTHA and BMIPP is depressed in ischemic myocardium; in hypoxic myocardium, increased retention of BMIPP was demonstrated, whereas FTHA retention was un-

changed. The reduction of retention in ischemia confirms the applicability of FTHA and BMIPP for determining fatty acid oxidation rates in ischemic myocardium. In hypoxic myocardium, both tracers displayed a lack of sensitivity for the change in beta-oxidation; the greater retention of BMIPP suggests greater dependence of the uptake mechanism on cytosolic metabolism (triacylglycerol synthesis or incorporation in lipid pools) of BMIPP than FTHA.

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