Myocardial Uptake of the Fatty Acid Analog 14-Fluorine-18-Fluoro-6-Thia-Heptadecanoic Acid in Comparison to Beta-Oxidation Rates by Tritiated Palmitate

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The fatty acid tracer 14-^{18}F-fluoro-6-thia-heptadecanoic acid (FTHA) is a metabolically trapped tracer of exogenous fatty acid utilization. The objectives of this study were to determine the relationship of FTHA uptake to changes in perfusion and fatty acid oxidation and to confirm the retention of FTHA in the mammalian heart. **Methods:** Six pigs with extracorporeal perfusion of the left anterior descending artery (LAD) and cannulation of the LAD vein were studied. The extraction fraction (EF) of FTHA, measured from LAD arterial and venous blood samples, was compared to beta-oxidation rates, determined by water production from tritiated palmitate. After a baseline period, changes in FTHA EF were measured in 15-min periods of hyperemia, control (baseline flow rate) and lactate infusion. After the lactate infusion, FTHA infusion was terminated, and a 15-min washout period was observed. **Results:** Beta-oxidation rate was unchanged from the baseline period during the hyperemic and control periods. With lactate infusion, the expected myocardial preference for lactate was noted, with a decline in exogenous fatty acid oxidation. Fluorine-18-FTHA EF paralleled the changes in beta-oxidation, with a decrease in EF during lactate infusion. Increase in perfusion was associated with a decrease in FTHA EF, compared to control, such that the product of flow and extraction was maintained. A linear relationship of FTHA EF to fractional tritiated water production was found. Washout analysis confirmed minimal washout of tracer at 15 min after termination of infusion. Organic solvent extraction of tissue samples suggested that the majority of tissue radioactivity was protein-bound. **Conclusion:** In the extracorporeally perfused mammalian heart, FTHA EF declined during suppression of beta-oxidation with lactate infusion and alteration in perfusion without change in fatty acid oxidation rate. The linear relationship of FTHA EF with fractional water production from tritiated palmitate further confirms a correlation of the uptake of FTHA with fatty acid beta-oxidation rate and supports the utility of FTHA in the noninvasive determination of fatty acid oxidation rate. Furthermore, the trapped nature of the tracer may allow the use of graphical analysis for the quantification of beta-oxidation rates.

Key Words: fatty acid tracer; 14-fluorine-18-fluoro-6-thia-heptadecanoic acid; myocardial metabolism; fatty acid metabolism; fatty acids

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Despite the role of the fatty acid as the main metabolic substrate for the heart, noninvasive determination of the rates of beta-oxidation with labeled fatty acid analogs has been less frequently used than the assessment of glucose metabolism with ^{18}F-fluorodeoxyglucose (FDG). Two different approaches in fatty acid tracer development have been used. With the first approach, fatty acid beta-oxidation rates have been estimated from the kinetics of tracers that are fully metabolized, with the rate of radioactivity loss from the heart being indicative of the beta-oxidation rate. Palmitate with ^{11}C substituted at the first carbon position is the most common example of such a tracer, allowing the assessment of myocardial clearance rates by PET after bolus administration of ^{11}C-palmitate (*1,2*). Recently, parametric modeling was used to determine beta-oxidation rates in dogs in a variety of metabolic settings (*3*). A second strategy has been the development of structurally altered fatty acid tracers in which uptake is proportional to beta-oxidation rates by means of metabolic trapping. This approach is analogous to the assessment of glucose phosphorylation rates with FDG. Fatty acids methylated in the beta position, such as 15-(p-iodophenyl)-3-(R,S)-methyl pentadecanoic acid (BMIPP), have been proposed, because the methyl group would block the beta-oxidation of such tracers. However, the sensitivity of such tracers to beta-oxidation remains to be demonstrated because the trapping process may be dominated by incorporation of the label into complex lipids (*4,5*).

Recently, a sulfur-substituted fatty acid, 14-^{18}F-fluoro-6-thia-heptadecanoic acid (FTHA), has been proposed as a trapped fatty acid analog (*6*). This analog has a sulfur heteroatom substitution to block beta-oxidation of the molecule (*7*) and a ^{18}F label to allow quantitative imaging with PET in vivo. Isolated murine heart experiments by DeGrado et al. (*8*) demonstrated a decrease in trapping rates of ^{18}F-FTHA after administration of a beta-oxidation inhibitor. Furthermore, the slow clearance of ^{18}F radioactivity from tissue after FTHA administration suggests that beta-oxidation rates may be quantitated in a manner similar to that used in FDG studies for determining glucose phosphorylation rates (*9*). However, as with FDG, it is critical to determine in a large mammal model, representative of the human, that the unidirectional uptake rate of FTHA reflects beta-oxidation and not some other intermediary physiologic process, such as capillary transit or incorporation in lipid pools. Thus, the primary goal of this work was to determine the tracer kinetics of a sulfur-substituted fatty acid analog (FTHA) in swine myocardium under two controlled experimental conditions. In the first condition, blood flow was increased without a change in fatty acid oxidation to determine the dependency of FTHA uptake on perfusion. In the second condition, circulating lactate levels were increased to suppress myocardial beta-oxidation through the known myocardial preference for lactate. A secondary goal was to confirm the retention of FTHA in the mammalian heart.

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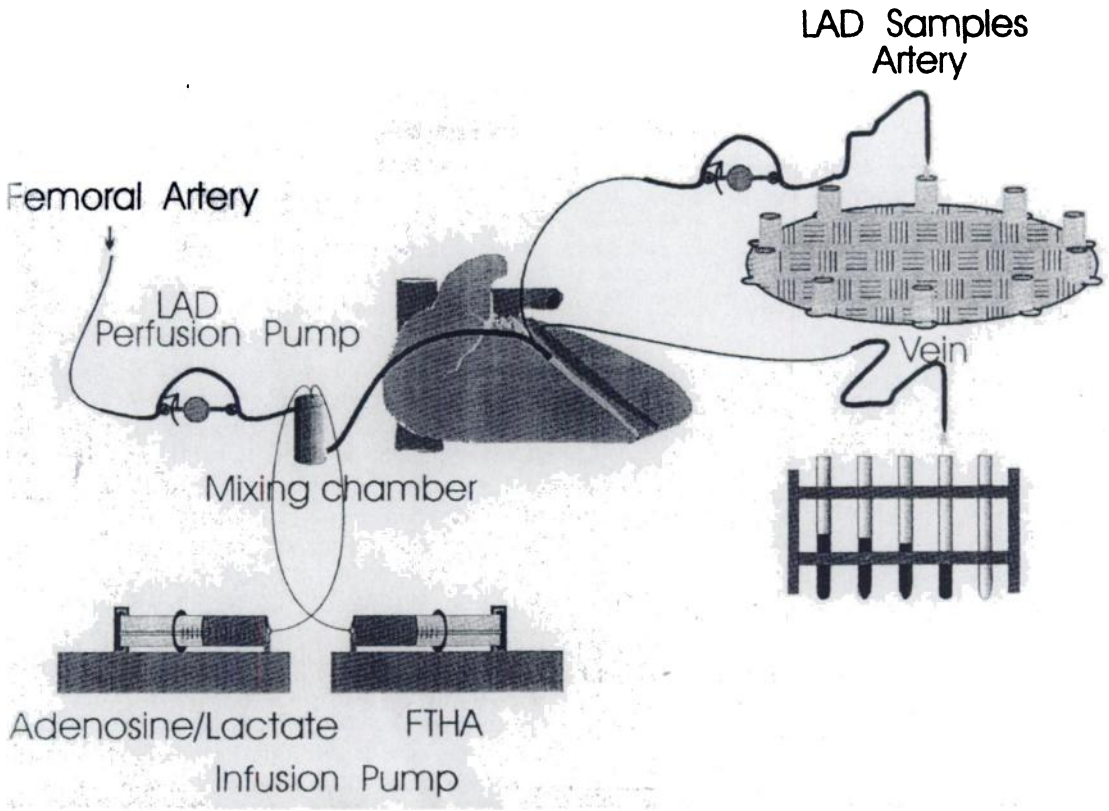

FIGURE 1. Experimental preparation is shown with extracorporeal perfusion of left anterior descending artery (LAD) with blood from femoral artery. For intervention states of hyperemia and lactate infusion, adenosine and lactate were infused directly into mixing chamber that was used to perfuse LAD. Tritiated palmitate was also infused into mixing chamber (not shown on diagram). LAD arterial and venous samples were obtained for measuring FTHA extraction fraction, myocardial oxygen consumption calculation and tritiated water production.

MATERIALS AND METHODS

Radiopharmaceutical Synthesis

Fluorine-18-FTHA was synthesized according to the method of DeGrado (*6*) using the precursor benzyl-14(R,S)-tosyloxy-6-thiaheptadecanoate. Fluorine-18-fluoride was produced by bombardment of ^{18}O target with 11-MeV protons in the Department of Medical Physics RDS Cyclotron (CTI, Inc., Knoxville, TN) (*10*). Reaction products in the precursor chemistry were purified by column chromatography and verified by nuclear magnetic resonance using a 400-MHz Bruker Spectrometer. Typical yields of the nucleophilic reaction of ^{18}F with benzyl-14(R,S)-tosyloxy-6-thiaheptadecanoate were 65%–75%, with final ^{18}F-FTHA yields after deprotection of 45%–55%. The product was purified by high-performance liquid chromatography (HPLC) using dual serial ultraviolet and gamma-ray detection. The ^{18}F-FTHA radioactivity peak is well isolated from any mass peak, resulting in carrier-free FTHA. Verification of the location of the ^{18}F-FTHA HPLC peak was accomplished by synthesizing a quantity of ^{19}F-FTHA sufficient for nuclear magnetic resonance verification and coinjecting this with previously HPLC-separated ^{18}F-FTHA. HPLC analysis resulted in simultaneous ultraviolet and gamma-ray peaks.

Surgical Preparation

The anesthesia and surgical preparation were reviewed and approved by the University of Wisconsin-Madison Research Animal Resource Committee. The preparation has been well established in the Cardiovascular Research Laboratory and had been used extensively in the investigation of myocardial metabolism in the ischemic and reperfused states (*11–15*). Anesthetic state was induced with a mixture of ketamine (11 mg/kg), atropine (0.8 mg) and acepromazine (1.1 mg/kg) intramuscularly. The initial anesthetic state was achieved with sodium thiopental (25 mg/kg) intravenously and maintained with alpha-chlorolose (1.5-g load and 0.5 g/hr intravenously) and subcutaneous morphine sulfate (45 mg/hr). A tracheostomy was performed on the swine (40–50 kg) in the supine position, and the pigs were ventilated through an endotracheal tube using 100% oxygen. The sternum was removed, as was the anterior rib cage. The right internal carotid artery and internal jugular vein were isolated, and a Millar catheter for measurement of aortic and left ventricular pressures and an intravenous line for medication and fluid administration were inserted. The femoral artery and vein were cannulated also. A pericardial cradle was constructed. The left anterior descending artery (LAD) was isolated and cannulated. The LAD was perfused from the femoral artery using a perfusion pump (Fig. 1). The LAD vein was cannulated and drained into the left axillary crevice. Suction was used to empty the crevice with return of the blood to circulation via the femoral vein. Thickness crystals were placed in the LAD bed for measuring mechanical performance.

Experimental Protocol

Arterial and venous blood samples were collected (every 10 min) for the determination of myocardial oxygen consumption. Rates of palmitate utilization were determined by infusion of 9,10-^{3}H-palmitate in the LAD via a mixing chamber, and periodic (every 10 min) assessment of arterial and venous samples for tritiated water was performed. Cardiogreen was infused into the same mixing chamber for 5 min at selected intervals, and samples were obtained to measure the dilution factor K. After stabilization for 30 min, the pig was given a 45-min infusion of the radiolabeled fatty acid analog FTHA at a rate of 68 ± 8 μCi/min for a total infusion of 3.1 ± 0.3 mCi (decay corrected to the start of infusion) via the mixing chamber for the LAD system. During administration of the radiolabeled fatty acid analog, blood samples were obtained

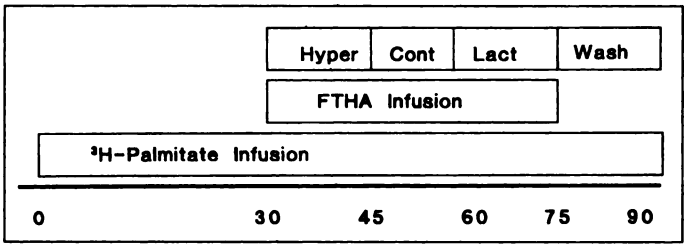

FIGURE 2. Protocol time line is depicted with 90-min infusion period for tritiated palmitate, 45-min infusion period for FTHA and 15-min states of hyperemia (Hyper), control (Cont) and lactate infusion (Lact) and 15-min washout period (Wash).

at 3-min intervals from the LAD arterial cannula and LAD venous sites for counting for ^{18}F radioactivity. During the FTHA infusion, uptake was measured during hyperemia, control conditions and lactate infusion (Fig. 2). Hyperemia was achieved by increasing LAD perfusion rates to a target level of a twofold increase in LAD blood flow. Adenosine was infused directly into the LAD arterial perfusion catheter to maintain perfusion pressure at baseline levels. After 15 min of hyperemia, LAD perfusion rate was returned to the control state for 15 min. During a third 15-min period, lactate was infused to achieve a final additive concentration in the LAD arterial system of 10 m*M* (*16*). Infusion of FTHA was then terminated, and LAD arterial and venous samples were collected over 15 min for washout analysis. The total duration of the experiment was 90 min. The animals were then euthanized with 100 mg/kg sodium pentobarbital. After euthanization, a 3- to 4-g myocardial tissue sample was resected from the LAD bed, rinsed in saline to remove blood and minced in saline for solvent extraction as described below; thereafter, the LAD bed was stained with India ink, resected, weighed and counted for ^{18}F radioactivity.

Biochemical Analyses

Arterial and venous blood samples were centrifuged and assayed for ^3H-H_2O according to the procedure of Saddik and Lopaschuk (*17*). Two aliquots from each plasma sample were assayed in duplicate. Organic solvent extraction of the plasma samples was performed with serial addition of chloroform/methanol (1:2), chloroform and 2 *M* potassium chloride/hydrogen chloride. After centrifugation, the aqueous layer was removed and then treated with a chloroform, methanol and potassium chloride/hydrogen chloride solution (1:1:0.9). The aqueous layer was counted for ^3H-H_2O in a Packard liquid scintillation counter with results reported as the average of the four values.

Fractional water production (FWP) was calculated as:

$$FWP = \frac{\Delta^3H_2O}{K \times ARA_{FFA}},$$

where ARA_{FFA} is the arterial radioactivity of 9,10-^3H-palmitate (counts/ml), K is the dilution factor and Δ^3H-H_2O is the arteriovenous concentration difference (counts/ml) of ^3H-H_2O. The dilution factor K was calculated as the ratio of venous to arterial cardiogreen concentration as measured by ultraviolet absorption. The beta-oxidation rate of free fatty acids was calculated from the arteriovenous difference of labeled water according to the formula:

$$OR_{FFA} = \frac{\Delta^3H_2O \times Q_{LAD}}{K \times ASA_{FFA} \times LAD_{drywt}},$$

where OR_{FFA} is the oxidation rate for free fatty acids (μmol/min/g dry weight), ASA_{FFA} is the arterial specific radioactivity of 9,10-^3H-palmitate (counts/μmol), LAD_{drywt} is the dry weight of the LAD perfusion bed (17% of the wet weight) (*18*) and Q_{LAD} is the LAD blood flow. Plasma fatty acid levels were determined using a colorimetric determination (NEFA-C; Wako Chemicals, Richmond, VA). Fluorine-18-FTHA extraction fraction (EF) was calculated for LAD arterial (A) and venous (V) ^{18}F radioactivity (counts/ml) and corrected for venous hemodilution by the dilution factor K:

$$FTHA\ EF = \frac{A - V/K}{A}.$$

Femoral arterial blood samples, obtained during each intervention period, had negligible radioactivity (<1% of LAD arterial radioactivity).

Blood and tissue samples were analyzed for the level of radioactivity in different lipid fractions of ^{18}F according to the method of Hamilton and Comai (*19*). Tissue aliquots (0.5 g) were homogenized in an organic solution of water, chloroform and methanol (0.8:1:2) and centrifuged. The chloroform layer was removed, and the precipitate was washed with chloroform. Sodium dodecyl sulfate extraction of the tissue was not performed because of the short half-life of the ^{18}F label. Both chloroform aliquots were combined and evaporated to dryness. After resuspension in 2.0 ml of hexane/tert-butyl methyl ether (MTBE) (200:3), 1.8 ml of the suspension was placed on a silica Sep-Pak column for chromatography. The remaining 0.2 ml was counted to determine the amount of radioactivity that was extracted. Elutions of the various fractions were performed with the column using 12-ml elutions of each of the following solvent systems: hexane/MTBE (200:3, "cholesterol ester" fraction), hexane/MTBE (96:4, "triglyceride" fraction), hexane/acetic acid (100:0.2, for acidification of the column with the eluate discarded), hexane/MTBE/acetic acid (100:2:0.2, "free fatty acid" fraction), MTBE/acetic acid (100:0.2, "cholesterol" fraction) and methanol ("phospholipid" fraction). Percent extraction of ^{18}F label from the precipitate was calculated by counting the radioactivity of the precipitate and an aliquot from the total lipid fraction before elution. From each of the eluted fractions, a 1-ml aliquot was counted for ^{18}F using a gamma counter while the volume of the remaining 11 ml was reduced and counted in the liquid scintillation counter after addition of liquid scintillation cocktail for ^3H radioactivity.

Statistical Analysis

Data from the six experiments were averaged, and the results were expressed as mean ± s.e.m. Data for the last 6 min of the baseline period and of each intervention period were compared using the paired two-tailed Student's t-test. Before testing for significance, Bonferroni correction for multiple comparisons was performed by multiplying the derived probability (p) values by the total number of comparisons (*2*). Differences with a $p < 0.05$ were assumed to be significant.

RESULTS

Changes in the hemodynamic parameters of heart rate, LV systolic pressure, pressure rate product and systolic thickening for the experimental periods of baseline, hyperemia, control, lactate and washout were not significant (Table 1). There was also no change in myocardial oxygen consumption throughout the protocol (Fig. 3). Similarly, stable dilution factor K values (baseline, 0.94 ± 0.02; hyperemia, 0.97 ± 0.02; control, 0.95 ± 0.02; lactate, 0.95 ± 0.02) and fatty acid levels (baseline, 0.46 ± 0.05 μmol/ml; hyperemia, 0.49 ± 0.07 μmol/ml; control, 0.44 ± 0.07 μmol/ml; lactate, 0.46 ± 0.07 μmol/ml) were noted.

Fatty acid oxidation rates were determined by tritiated water production, determined from LAD arterial and venous blood samples. Despite a trend toward an increase in fatty acid oxidation during hyperemia, no significant change in fatty acid oxidation rates was noted during the baseline, hyperemia and

TABLE 1
Hemodynamic Parameters During Baseline, Hyperemia, Control, Lactate Infusion and Washout

Experimental period	Heart rate (bpm)	Left ventricular systolic pressure (mm Hg)	Pressure rate product (mm Hg·bpm × 1000)	% systolic thickening*
Baseline	128 ± 12	79 ± 6	10 ± 1	1
Hyperemia	138 ± 15	80 ± 6	11 ± 1	1.0 ± 0.2
Control	140 ± 14	85 ± 9	12 ± 2	0.8 ± 0.1
Lactate	140 ± 20	76 ± 11	11 ± 2	0.7 ± 0.3
Washout	145 ± 22	90 ± 3	13 ± 2	0.8 ± 0.2

*Systolic thickening is expressed as a ratio to the baseline period.

control periods (0.29 ± 0.06, 0.43 ± 0.08 and 0.35 ± 0.07 μmol/min/g dry weight, respectively; Figs. 4 and 5). However, a significant decrease in fatty acid oxidation rate was noted with lactate infusion (0.11 ± 0.03, $p < 0.01$ compared to control). Fractional water production tended to decline during hyperemia and lactate infusion (0.07 ± 0.01 and 0.04 ± 0.01, respectively, versus 0.12 ± 0.03 for baseline and 0.12 ± 0.03 for control), although the decrements did not make statistical significance ($p < 0.10$). Fluorine-18-FTHA EF increased during the control period (0.24 ± 0.03) compared to the hyperemia period (0.12 ± 0.02, $p < 0.01$) and declined again during lactate infusion (0.11 ± 0.01, $p < 0.04$). A linear relationship between fractional water production and FTHA EF was noted (Fig. 6); the linear regression of fractional water production versus FTHA EF was $y = 1.07x + 0.0868$, ($r = 0.80$, $p < 0.001$).

The washout of radioactivity from the LAD perfusion bed was also determined. LAD arterial and venous samples were taken at 1, 3, 5 and 15 min after cessation of the FTHA infusion. Both arterial and venous concentrations declined sharply as the tracer cleared from the mixing chamber and arterial perfusion system. By 15-min postinfusion, the difference between venous and arterial radioactivity concentrations, reflecting tissue washout, had declined to 0.7% ± 0.1% of the mean tissue radioactivity concentration in the LAD bed, determined at the end of the experiment. This small clearance rate is consistent with clearance half-times of one to several hours for the trapped tracer. Analysis of the deposition of FTHA was performed on homogenized myocardial tissue samples from the LAD perfusion bed at the end of the experiment, using organic solvent extraction of various lipid components. The majority of ^{18}F radioactivity was in the protein precipitate (79% ± 10%) and

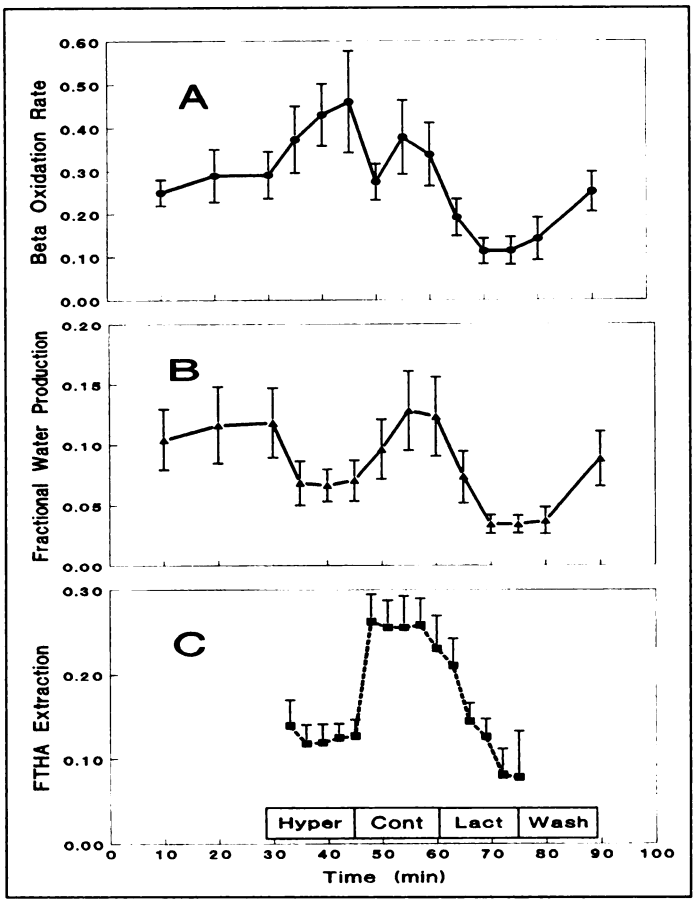

FIGURE 4. Beta-oxidation rate (A, μmol/min/g dry weight), fractional water production (B, no units) and FTHA EF (C, no units) are shown for left anterior descending artery (LAD) perfusion bed. Beta-oxidation rate and fractional water production were calculated from LAD arteriovenous differences of tritiated water. Net FTHA extraction was calculated as LAD arteriovenous difference of ^{18}F radioactivity normalized to arterial ^{18}F radioactivity. Time interval abbreviations are defined in legend to Figure 2.

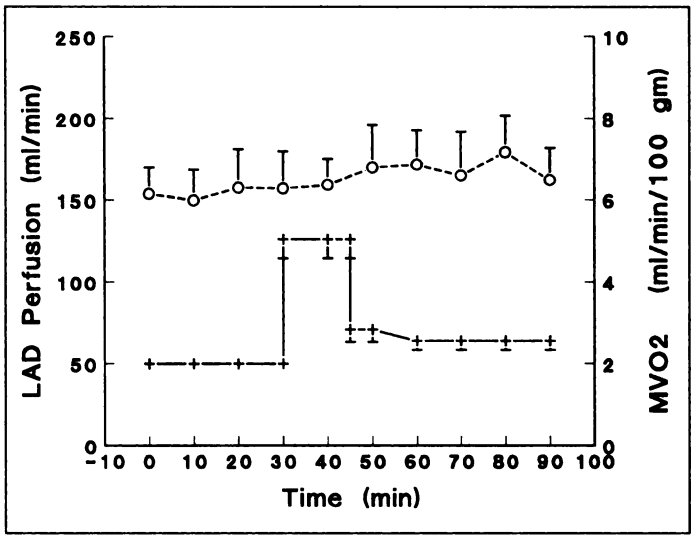

FIGURE 3. Left anterior descending artery (LAD) perfusion rate (+) and myocardial oxygen consumption (MVO_2, ○) in LAD perfusion bed is shown throughout experiment for all six animals. No change in myocardial oxygen consumption was seen throughout experimental protocol.

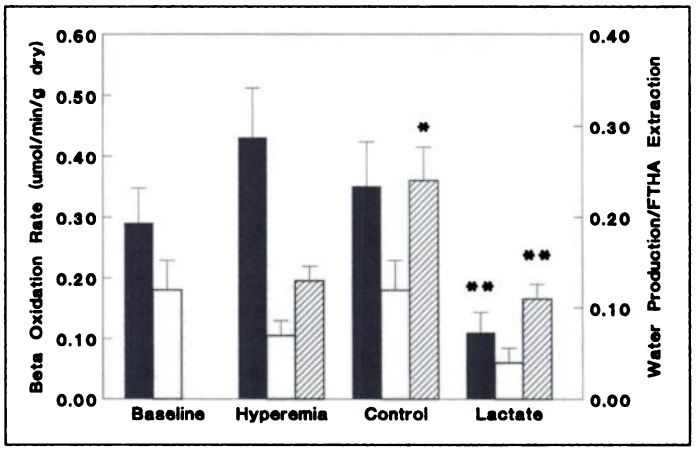

FIGURE 5. Comparison of beta-oxidation rate (solid bar, μmol/min/g dry weight), fractional water production (open bar, no units) and FTHA extraction fraction (EF) (cross-hatched bar, no units) for last 6 min of baseline, adenosine infusion, control and lactate infusion periods. (*$p < 0.05$ versus hyperemia; **$p < 0.05$ versus control). A significant decrease in beta-oxidation rate occurred with lactate infusion. Fluorine-18-FTHA EF increased during control period compared to hyperemia and decreased in lactate infusion period.

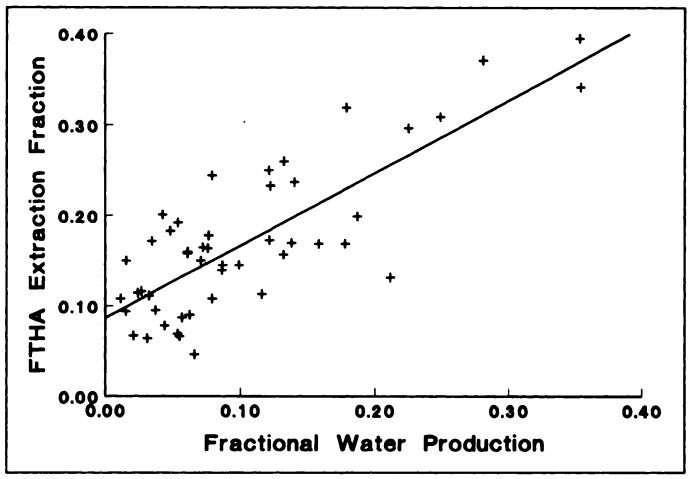

FIGURE 6. Fluorine-18-FTHA extraction fraction (EF) versus fractional water production. Linear relationship of FTHA EF versus fractional water production was seen. Equation for linear regression was $y = 1.07x + 0.0868$ ($r = 0.80$, $p < 0.001$).

could not be extracted. The portion of ^{18}F radioactivity that could be extracted was divided between the aqueous phase (8% ± 6%) and the organic layer (13% ± 6%). These results were in contrast to fractionation of FTHA added to blood and saline in vitro. Fractionation of blood with FTHA added in vitro demonstrated negligible radioactivity in the precipitate, 97.8% of the radioactivity in the phospholipid fraction, 1.4% in the cholesterol fraction and no radioactivity in the cholesterol ester, triglyceride or free fatty acid fractions. A similar fractionation of FTHA radioactivity added to saline in vitro was seen with 94.6% ± 0.2% in the phospholipid fraction and 5.0% ± 0.2% in the cholesterol fraction. Fluorine-18-FTHA added to tissue samples in vitro was 95.3% ± 0.1% in the organic phase with only 4.2% ± 0.1% in the precipitate.

DISCUSSION

The goal of this experiment was to determine the utility of FTHA as a retained tracer of myocardial fatty acid oxidation rates. A 30-min baseline period was used to allow for adequate loading of cytoplasmic lipid pools with ^{3}H-palmitate. Thereafter, each pig underwent three different experimental conditions of hyperemia to assess the flow dependency of FTHA uptake, baseline perfusion rate (control) and baseline perfusion rate with lactate infusion to suppress fatty oxidation via the known preference of the myocardium for lactate over fatty acids as a metabolic substrate. The results confirm that the uptake of FTHA is sensitive to changes in the rate of beta-oxidation and not perfusion. The increase in LAD blood flow with maintenance of baseline pressure was not associated with a significant change in beta-oxidation rate. Although a decline in fractional water production with the increase in tracer delivery was anticipated, the trend noted in fractional water production with hyperemia was not significantly depressed. However, ^{18}F-FTHA EF decreased in inverse proportion to the increase in blood flow, such that the product of flow and extraction was maintained. Lactate infusion was associated with a decline in beta-oxidation rate, consistent with the shift in myocardial metabolism from fatty acids to lactate. Fluorine-18-FTHA extraction was correspondingly sensitive to this change in metabolism, demonstrating a decrease in EF. These results suggest that the net extraction of FTHA is sensitive to beta-oxidation rates and that the uptake is not flow related. This sensitivity was further supported with the linear relationship of FTHA EF to fractional water production.

The majority of the experimental time was used for the intervention studies, leaving a 15-min washout period that was too brief to allow the washout half-life to be quantitatively evaluated. However, the minimal loss during washout is consistent with a lower limit of at least 1 hr for the washout half-time of the trapped tracer. Future experiments with a longer time frame for the determination of change in tissue and venous radioactivity are needed to define the washout kinetics. Furthermore, in isolated rat heart (*20*), linear uptake over 35 min was noted with constant infusion of tracer, confirming that FTHA is a retained tracer. Analysis of tissue samples for the deposition of FTHA is also consistent with the metabolism of the tracer in that the majority of tracer radioactivity could not be extracted from the precipitate, in contrast to FTHA added in vitro. However, the positive intercept of the linear regression of FTHA EF versus fractional water production suggests that, at lower levels of beta-oxidation, not all FTHA binding is related to oxidative metabolism. It is possible, at these lower levels of beta-oxidation, that FTHA is incorporated in lipid pools or preferentially oxidized. Future experiments are planned to localize the deposition of FTHA to cytosolic or mitochondrial fractions.

The design and development of a fatty acid tracer is motivated by the central nature of exogenous fatty acids as a metabolic substrate for the myocardium. A variety of circulating and endogenous substrates including fatty acids, glucose, lactate, ketones and amino acids serve as carbon units for cardiac myocyte oxidative phosphorylation for the regeneration of adenosine triphosphate. Fatty acids in the normal heart are the preferred substrate, accounting for 60%–70% of the energy production. Furthermore, although generation of fatty acids from endogenous lipid stores is possible (*21*), the majority of metabolized fatty acids is derived from circulating fatty acids with avid first-pass extraction of 55% of nonesterified fatty acids occurring based on arteriovenous differences (*22*). The complexity of fatty acid metabolism, however, has significantly hindered the development of a tracer, the retention of which is related to beta-oxidation rates. Several intermediary metabolic steps in the cytosol and mitochondria are involved. In addition, free fatty acid may be incorporated into cytoplasmic lipid pools and not immediately undergo metabolism.

Determination of fatty acid utilization rates has been performed in the laboratory and clinical settings using a variety of methods and tracers. Classical myocardial metabolic studies have used arteriovenous differences of reaction end product concentrations to determine the rates of fatty acid oxidation. The most commonly used technique has been the measurement of the arteriovenous difference of ^{14}C-CO_2 after ^{14}C-palmitate infusion. Carbon-14-CO_2 is formed with the beta-oxidation of labeled palmitate and the oxidation of labeled acetyl-CoA. Concern has been expressed recently about the use of this technique because the incorporation of label in intermediate carbon pools may cause the rate of beta-oxidation determined with ^{14}C-palmitate to be underestimated (*23,24*). The use of 9,10-^{3}H-palmitate has been advocated by some investigators, with the release of tritiated water being a more constant proportion of the total tritiated byproducts released (*25*). With beta-oxidation, 75% of the tritium from 9,10-^{3}H-palmitate will be released as tritiated water with the remainder as 2-^{3}H-acetyl intermediates. Because the metabolism of these intermediates is variable, the measurement of tritiated water production has been proposed as a more reproducible measurement of beta-oxidation of long chain fatty acids.

Most clinical studies have instead used noninvasive radionuclide imaging techniques because of the difficulty in obtaining

LAD venous samples clinically. Several studies have used PET with ^{11}C-palmitate in determination of beta-oxidation rates. Schön et al. (*2*) at the University of California at Los Angeles performed the initial investigations of palmitate kinetics in vivo with PET. Dynamic imaging was performed of ^{11}C-palmitate uptake and washout. Washout rate analysis was used to determine K_1, the rate of loss of ^{11}C, in the form of $^{11}C\text{-}CO_2$, from the heart. Changes in K_1 were noted that paralleled the increase in the workload of the heart. Similarly, reduction in flow was associated with a decrease in clearance, suggestive of reduced oxidative rates (*26*). A relative prolongation in clearance times was also seen with tachypacing-induced ischemia (*27*). Recently, parametric modeling of ^{11}C-palmitate activity in dogs by Bergmann et al. (*3*) has confirmed the changes in beta-oxidation rate with changes in workload or perfusion.

Several iodinated fatty acids have been advocated for the assessment of fatty acid oxidation rates using single-photon imaging. The most widely studied iodinated tracer based on a metabolic clearance design is ^{123}I-phenylpentadecanoic acid (*28*). In addition, a variety of trapped tracers have been developed (*29–32*) that have excellent myocardial uptake and target-to-background ratios. These tracers have been modified such that their oxidation is blocked by structural substitutions such as a tellurium heteroatom (*29*) or a methyl group (*30–34*), causing the tracer to be retained. However, studies have demonstrated that the uptake of 15-(p-iodophenyl)-6-tellurapentadecanoic acid closely paralleled blood flow (*35,36*). Furthermore, recent isolated rat heart data have demonstrated the insensitivity of BMIPP uptake to the blockade of beta-oxidation. Thus, uptake of BMIPP is not reflective of beta-oxidation rates (*5*).

Limitations of this study are related to the experimental design. The use of three serial conditions allowed for a paired comparison of experimental variables but did not allow for the testing of whether differences in myocyte handling of FTHA existed in the different conditions. We were able to determine the localization of radioactivity at the completion of the experiment, which represented the combined deposition of the hyperemia, control and lactate periods. Although a correlation of FTHA EF with fractional water production was demonstrated, a positive intercept was also found with the linear regression, suggesting less of a decline in FTHA EF at lower rates of beta-oxidation. The current experimental design did not allow for a comparison of radioactivity deposition between the control and lactate infusion conditions. Future studies are needed to define the cellular metabolism of FTHA in which animals will be subjected to only one experimental condition to allow comparison of the deposition of radioactivity between groups. Furthermore, the current study of LAD bed radioactivity confirmed the presence of a large fraction in the pellet consistent with protein binding. However, not clarified by this work is whether the binding was within the mitochondrial or cytosolic fractions. Separation of the binding in subcellular fractions, as well as determination of the labeled protein size, could also be accomplished with differential centrifugation and protein gel electrophoresis. Here, the metabolite fraction was confirmed to be small with no significant accumulation of radioactivity in the femoral arterial blood samples. However, in vivo injections of FTHA will require measurement of metabolites and correction of the input function if significant metabolite formation is present.

A primary experimental need exists for a tracer of beta-oxidation rates that could be used to assess changes in fatty acid metabolism in different cardiac physiologic and pathologic states. PET allows the performance of quantitative imaging in vivo, allowing this assessment in intact animals or patients. Imaging the heart with a retained tracer of fatty acid metabolism may represent a simplification, compared to labeled palmitate. Further clinical investigations with a tracer of beta-oxidation is warranted from research and clinical perspectives. From a research perspective, current understanding of myocardial metabolism in several pathologic conditions is limited by the lack of data concerning the handling of the major metabolic substrate of the heart. From a clinical perspective, there are several instances when quantitative assessment of beta-oxidation would be important. On the basis of the available animal literature, myocardial ischemia is known to have a significant inhibitory effect on fatty acid metabolism. Thus, a fatty acid tracer may be a sensitive indicator of extent of myocardial ischemia. Second, in the cardiomyopathic patients, changes in myocardial fatty acid oxidation are thought to occur (*37,38*). The determination of the alteration of beta-oxidation rates may identify patients with reduced myocardial metabolic reserve who may benefit from earlier transplantation. Further studies are needed clinically with a tracer of beta-oxidation to substantiate this hypothesis.

CONCLUSION

The myocardial uptake of the long-chain fatty acid tracer FTHA declined with reduction in beta-oxidation rate with elevation in plasma lactate levels. Similarly, FTHA EF declined during hyperemia in a manner that was inversely proportional to the flow increment. These results are consistent with the correspondence of FTHA myocardial uptake to the beta-oxidation rate. This relationship was further demonstrated by the linear correlation of FTHA EF with myocardial fractional water production from tritiated palmitate infusion. Further studies are needed in other myocardial metabolic states such as ischemia to determine the utility of FTHA as a retained fatty acid tracer.

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REFERENCES

1. Schön HR, Schelbert HR, Najafi A, et al. C-11 labeled palmitic acid for the noninvasive evaluation of regional myocardial fatty acid metabolism with positron-computed tomography. I. Kinetics of C-11 palmitic acid in normal myocardium. *Am Heart J* 1982;103:532–547.
2. Schön HR, Schelbert HR, Najafi A, et al. C-11 labeled palmitic acid for the noninvasive evaluation of regional myocardial fatty acid metabolism with positron-computed tomography. II. Kinetics of C-11 palmitic acid in acutely ischemic myocardium. *Am Heart J* 1982;103:548–561.
3. Bergmann SR, Weinheimer CJ, Markham J, Herrero P. Quantitation of myocardial fatty acid metabolism using PET. *J Nucl Med* 1996;37:1723–1730.
4. DeGrado TR, Holden JE, Ng CK, Raffel DM, Gatley SJ. Comparison of 16-iodohexadeanoic acid (IHDA) and 15-p-iodophenylpentadecanoic acid (IPPA) metabolism and kinetics in the isolated rat heart. *Eur J Nucl Med* 1988;14:600–606.

5. DeGrado TR, Holden JE, Ng CK, Raffel DM, Gatley SJ. β-Methyl-15-p-iodophenylpentadecanoic acid metabolism and kinetics in the isolated rat heart. *Eur J Nucl Med* 1989;15:78–80.
6. DeGrado TR. Synthesis of 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA). *J Labeled Comp Radiopharm* 1991;29:989–995.
7. Skrede S, Narce M, Bergseth S, Bremer J. The effects of alkylthioacetic acids (3-thia fatty acid) on fatty acid metabolism in isolated hepatocytes. *Biochim Biophys Acta* 1989;1005:296–302.
8. DeGrado TR, Coenen HH, Stocklin G. 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA): evaluation in mouse of a new probe of myocardial utilization of long chain fatty acids. *J Nucl Med* 1991;32:1888–1896.
9. Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 1983;3:1–7.
10. Nickles RJ, Gatley SJ, Votaw JR, Kornguth ML. Production of reactive fluorine-18. *Int J Radiat Appl Instrum Part A* 1986;37:649–661.
11. Liedtke AJ, Renstrom B, Nellis SH. Correlation between [5-^{3}H]glucose and [U-^{14}C]deoxyglucose as markers of glycolysis in reperfused myocardium. *Circ Res* 1992;71:689–700.
12. Renstrom B, Nellis SH, Liedtke AJ. Metabolic oxidation of glucose during early myocardial reperfusion. *Circ Res* 1989;65:1094–1101.
13. Liedtke AJ, DeMaison L, Eggleston AE, Cohen LM, Nellis SH. Changes in substrate metabolism and effects of excess fatty acids in reperfused myocardium. *Circ Res* 1988;62:535–542.
14. Renstrom B, Nellis SH, Liedtke AJ. Metabolic oxidation of pyruvate and lactate during early myocardial reperfusion. *Circ Res* 1990;66:282–288.
15. Nellis SH, Liedtke AJ, Renstrom B. Distribution of carbon flux within fatty acid utilization during myocardial ischemia and reperfusion. *Circ Res* 1991;69:779–790.
16. Rose CP, Goresky CA. Constraints on the uptake of labeled palmitate by the heart. The barriers at the capillary and sarcolemmal surfaces and the control of intracellular sequestration. *Circ Res* 1977;41:534–545.
17. Saddik M, Lopaschuk G. Myocardial triglyceride turnover during reperfusion of isolated rat hearts subjected to a transient period of global ischemia. *J Biol Chem* 1992;267:3825–3831.
18. Abu-Erreish GM, Neely JR, Whitmer JT, Whitman V, Sanadi DR. Fatty acid oxidation by isolated perfused working hearts of aged rats. *Am J Physiol* 1977;232:E258–E262.
19. Hamilton JG, Comai K. Rapid separation of neutral lipids, free fatty acids and polar lipids using prepacked silica Sep-Pak columns. *Lipids* 1988;23:1146–1149.
20. Pooley RA. Evaluation of 14(R,S)[fluorine-18]fluoro-6-thia-heptadecanoic acid (FTHA) as a positron emission tomography fatty acid tracer of β-oxidation in the heart. [PhD thesis]. Madison, WI: University of Wisconsin-Madison; 1995.
21. Gandemer GG, Durand G, Pascal G. Relative contribution of the main tissues and organs to body fatty acid synthesis in the rat. *Lipids* 1983;18:223–228.
22. Van der Vusse GJ, Roemen THM, Flameng W, Reneman RS. Serum myocardium gradients of nonesterified fatty acids in the dog, rat and man. *Biochem Biophys Acta* 1983;752:361–370.
23. Veerkamp JH, Van Moerkerk HTB, Glatz JFC, Zuurveld JGEM, Jacobs AEM, Wagenmakers AJM. $^{14}CO_2$ production is no adequate measure of [^{14}C]fatty acid oxidation. *Biochem Med Metab Biol* 1986;35:248–259.
24. Sherratt HSA, Watmough NJ, Johnson MA, Turnbull DM. Methods for study of normal and abnormal skeletal muscle mitochondria. *Methods Biochem Anal* 1988;33:243–335.
25. Kler RS, Sherratt HSA, Turnbull DM. The measurement of mitochondrial β-oxidation by release of $^{3}H_2O$ from [9,10-^{3}H]hexadecanoate: application to skeletal muscle and the use of inhibitors as models of metabolic disease. *Biochem Med Metab Biol* 1992;47:145–156.
26. Lerch RA, Ambos HD, Bergmann SR, Sobel BE, Ter-Pogossian MM, Sobel BE. Effect of flow-independent reduction of metabolism on regional myocardial clearance of ^{11}C-palmitate. *Circulation* 1982;65:731–738.
27. Grover-McKay M, Schelbert HR, Schwaiger M, et al. Identification of impaired metabolic reserve by atrial pacing in patients with significant coronary artery stenosis. *Circulation* 1986;74:281–292.
28. Hansen CL, Jeo J, Oliner C, Van Decker W, Iskandrian AS. Prediction of improvement in left ventricular function with iodine-123-IPPA after coronary revascularization. *J Nucl Med* 1995;36:1987–1993.
29. Goodman MM, Knapp FF Jr, Callahan AP, Ferren LA. A new well-retained myocardial imaging agent: radioiodinated 15-(p-iodophenyl)-6-tellurapentadecanoic acid. *J Nucl Med* 1982;23:904–908.
30. Fagret D, Bontemps L, Apparu M, et al. Kinetics of iodomethylated hexadecanoic acid metabolism in the rat myocardium: influence of the number and the position of methyl radicals. *Int J Nucl Med Biol* 1985;12:363–367.
31. Chouraqui P, Maddahi J, Henkin R, Karesh SM, Galie E, Berman DS. Comparison of myocardial imaging with iodine-123-iodophenyl-9-methyl pentadecanoic acid and thallium-201-chloride for assessment of patients with exercise-induced myocardial ischemia. *J Nucl Med* 1991;32:447–452.
32. Sloof GW, Visser FC, Eenige van MJ, et al. Comparison of uptake, oxidation and lipid distribution of 17-iodoheptadecanoic acid, 15-(p-iodophenyl)pentadecanoic acid and 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid in normal canine myocardium. *J Nucl Med* 1993;34:649–657.
33. Knapp FF, Ambrose KR, Goodman MM. New radioiodinated methyl-branched fatty acids for cardiac studies. *Eur J Nucl Med* 1986;12(suppl):S39–S44.
34. Nishimura T, Sago M, Kihara K, et al. Fatty acid myocardial imaging using ^{123}I-beta-methyl-iodophenyl pentadecanoic acid (BMIPP): comparison of myocardial perfusion and fatty acid utilization in canine myocardial infarction (occlusion and reperfusion model). *Eur J Nucl Med* 1989;15:341–345.
35. Bianco JA, Pape LA, Alpert JS, et al. Accumulation of radioiodinated 15-(p-iodophenyl)-6-tellurapentadecanoic acid in ischemic myocardium during acute coronary occlusion and reperfusion. *J Am Coll Cardiol* 1984;4:80–87.
36. Poe ND, Robinson GD, Graham SL, MacDonald NS. Experimental basis for myocardial imaging with ^{123}I-labeled hexadecanoic acid. *J Nucl Med* 1977;17:1077–1082.
37. Geltman EM, Smith JL, Beecher D, Ludbrook PA, Ter-Pogossian MM, Sobel BE. Altered regional myocardial metabolism in congestive cardiomyopathy detected by positron tomography. *Am J Med* 1983;74:773–785.
38. Eisenberg JD, Sobel BE, Geltman EM. Differentiation of ischemic from nonischemic cardiomyopathy with positron emission tomography. *Am J Cardiol* 1987;59:1410–1414.

Generation of Myocardial Factor Images Directly from the Dynamic Oxygen-15-Water Scan Without Use of an Oxygen-15-Carbon Monoxide Blood-Pool Scan

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The measurement of regional myocardial blood flow (MBF) with $H_2{}^{15}O$ and PET requires an additional $C^{15}O$ blood-pool scan for the purpose of region of interest (ROI) definition. This additional scan results in a substantially increased radiation dose, study duration and risk of movement artifacts. Therefore, a method was developed to generate myocardial factor images directly from the dynamic $H_2{}^{15}O$ study without the need for a $C^{15}O$ scan. **Methods:** The factor sinograms were generated by means of linear dimension reduction of the dynamic sinograms, where the required variate and covariate factors (myocardial and blood time-activity curves) were modeled from the lung time-activity curve. The factor images were generated by iterative reconstruction. **Results:** No significant difference was found between MBF values from ROIs drawn on the traditional images (using the $C^{15}O$ scan) and those drawn on the factor images. **Conclusion:** It is possible to generate myocardial images directly from the dynamic $H_2{}^{15}O$ study, so that the $C^{15}O$ scan can be omitted from MBF studies. The proposed method is robust and results in nearly optimal signal-to-noise ratios in the factor images.

Key Words: PET; myocardial blood flow; factor analysis; myocardial and blood-pool images

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The measurement of regional myocardial blood flow (MBF) with $H_2{}^{15}O$ and PET is an established technique based on the favorable properties of $H_2{}^{15}O$ as a flow tracer (*1–3*). The main

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