Metabolism of Radioiodinated Fatty Acid Analogs in Ischemic and Hypoxic Canine Myocardium

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Myocardial metabolism of 17-[^{123}I]-iodoheptadecanoic acid (IHDA), 15-(p-[^{131}I]-iodophenyl)pentadecanoic acid (pIPPA) and 15-(p-[^{125}I]-iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPP) was assessed during ischemia and hypoxia. The simultaneous investigation allowed us to evaluate differences in metabolic handling of these three fatty acids. **Methods:** In 17 open-chest dogs, the left ascending coronary artery was cannulated and extracorporeal bypass (ECB) perfused. In 3 dogs, ECB flow was kept normal, and these control experiments showed that kinetics of the radioiodinated fatty acids were not affected by the ECB technique itself. In 9 dogs, ECB flow was reduced to one third (ischemia), and in 5 dogs, the ECB area was perfused with venous blood and was kept at control values (hypoxia). After simultaneous intraveneous injection of IHDA, pIPPA and DMIPP, seven paired biopsy specimens from the native and ECB-perfused myocardium were taken over an assay period of 35 min. Total activity and the distribution in the aqueous phase and lipid fractions were determined, and time-activity curves were constructed. **Results:** In ischemic (Is) but not in hypoxic (Hy) myocardium, peak total activity of IHDA, pIPPA and DMIPP decreased significantly versus normal (N) myocardium (IHDA: N = 700 ± 267 versus Is = 335 ± 158 dpm/mg/mCi; pIPPA: N = 988 ± 318 versus Is = 438 ± 180 dpm/mg/mCi; DMIPP: N = 352 ± 146 versus Is = 179 ± 82 dpm/mg/mCi; all P values < 0.001). The relative decrease was similar for IHDA, pIPPA or DMIPP. Half-time values of total activity were prolonged for IHDA and pIPPA but were shortened for DMIPP in ischemic and hypoxic myocardium (IHDA: N = 22, Is = 44 and Hy = 50 min; pIPPA: N = 24, Is = 95 and Hy = 169 min; DMIPP: N = 528, Is = 409 and Hy = 115 min). The aqueous phase activity for IHDA, pIPPA and DMIPP decreased significantly versus normal myocardium in both ischemic (IHDA: N = 71% ± 9% versus Is = 36% ± 9%, $P < 0.001$; pIPPA: N = 62% ± 10% versus Is = 25% ± 8%, $P < 0.001$; DMIPP: N = 26% ± 11% versus Is = 18% ± 3%, $P < 0.05$) and hypoxic (IHDA: N = 76% ± 8% versus Hy = 62% ± 8%, $P < 0.05$; pIPPA: N = 66% ± 8% versus Hy = 46% ± 10%, $P < 0.05$; DMIPP: N = 32% ± 6% versus Hy = 24% ± 4%, $P < 0.05$) myocardium. The relative decrease was significantly highest for pIPPA and lowest for DMIPP. Incorporation into triacylglycerols increased significantly for IHDA, pIPPA and DMIPP in both ischemic and hypoxic myocardium. In normal myocardium, DMIPP was already mainly incorporated into triacylglycerols. Activity of IHDA and pIPPA in acylcarnitine increased significantly in ischemic and hypoxic myocardium. **Conclusion:** Kinetics of the radioiodinated fatty acid analogs in myocardium are altered during oxygen deprivation in a similar fashion as documented in literature for natural fatty acids. However, the changes were different between IHDA, pIPPA and DMIPP, suggesting different metabolic handling and thus reflecting different aspects of myocardial fatty acid metabolism.

Key Words: fatty acids; myocardial ischemia

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Under physiological conditions, long-chain fatty acids are the main source for oxidative myocardial energy production (*1*). However, in ischemia, fatty acid metabolism is profoundly altered. The absolute alterations are influenced by variables such as the severity and duration of oxygen restriction, the level of residual flow and alternative substrate availability. In the acute stage of oxygen deprivation, extraction of fatty acids is most likely reduced. Consistent with the expected effects of ischemia, the mitochondrial oxidation of fatty acids decreases, leading to accumulation of intermediates such as hydroxy fatty acids, acyl-CoA and acylcarnitine. A disproportionately larger fraction of fatty acid extracted is diverted into nonoxidative pathways, mainly into the triacylglycerol-fatty acid cycle (*2–4*).

A variety of fatty acid analogs have been developed to study myocardial fatty acid metabolism noninvasively (*5,6*). ^{123}I has favorable physical properties that allow acquisition with conventional gamma cameras. In recent years, both basic and clinical research have therefore focused on radioiodinated fatty acids (IFAs). An example of an aliphatic fatty acid analog is 17-iodoheptadecanoic acid (IHDA) with iodine in the omega position (Fig. 1). After oxidation, the radioiodine is released, and the free radioiodine leaves the cell and enters the blood, resulting in low myocardium-to-blood ratios. Also, nonspecific metabolic deiodination of IHDA may occur (*7*). The terminal iodophenyl-substituted fatty acid analog, 15-(p-iodophenyl)pentadecanoic acid (pIPPA) (Fig. 1), was introduced by Machulla et al. (*8*) to stabilize the radioiodine and to overcome possible nonspe-

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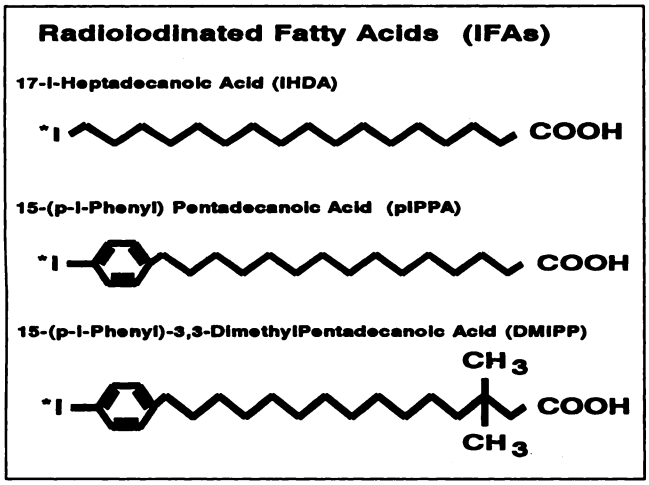

FIGURE 1. Structural formulas of IHDA, pIPPA and DMIPP.

cific deiodination. pIPPA is catabolized to para-iodobenzoic acid and is eliminated by the kidneys and the liver (*9*).

Introduction of methyl branching has been successful in increasing myocardial retention, allowing the assessment of regional myocardial uptake by SPECT. The 3-monomethyl-substituted analog, 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP), exhibits myocardial clearance slow enough to permit regional distribution studies by SPECT (*5,6*). However, because of catabolism by α-oxidation, followed by cycles of β-oxidation, considerable myocardial clearance still exists (*10–12*). To inhibit catabolism even more effectively, Knapp et al. (*10*) developed a dimethyl fatty acid analog, 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPP) (Fig. 1).

Although the aforementioned IFAs have been advocated as markers for altered fatty acid metabolism in myocardial ischemia, simultaneous investigation of myocardial metabolism of IHDA, pIPPA and DMIPP (key examples of iodoakyl-, iodophenyl- and methyl-branched fatty acid analogs, respectively) during ischemia has not yet been performed. Accordingly, the metabolic handling of these IFAs was studied over time by myocardial biopsies taken in an extracorporeal bypass (ECB)-perfused open-chest dog model. Because oxygen deprivation during ischemia is accompanied by inadequate washout of metabolites consequent to reduced perfusion, we also studied the metabolism of IFAs during hypoxia (i.e., decreased supply of oxygen at normal coronary perfusion).

The aim of this study was to assess the myocardial metabolism of IHDA, pIPPA and DMIPP during oxygen deprivation, regardless of concomitant reduction of perfusion. The observations will be compared with the metabolism of natural fatty acids described in the literature. In addition, the relative merits and drawbacks for fatty acid scintigraphy will be discussed.

MATERIALS AND METHODS

Of the 20 male mongrel dogs initially included in the study group, 3 were excluded because of cardiac arrest during the experiment (n = 2) or dysfunction of instrumentation (n = 1). Thus, 17 male mongrel dogs (27 ± 4 kg) were included in this study. After overnight fasting and premedication with 2.0–4.0 mL Thalamonal (Janssen-Cilag BV, Tilburg, The Netherlands) injected intramuscularly (2.5 mg droperidol/50 μg fentanyl/mL), anesthesia was induced by fentanyl (20 μg/kg) through a cannula in a cephalic vein, immediately followed by administration of etomidate (0.4 mg/kg). Before thoracotomy, droperidol (0.5 mg/kg) and fentanyl (40 μg/kg) were administered by bolus injection, followed by continuous infusion of fentanyl (10–20 μg/kg/h). The dogs were ventilated with N_2O/O_2 (2:1 by volume) and muscle relaxation was maintained with intravenous pancuronium (0.2 mg/kg before intubation, followed by 0.01 mg/kg/h). Arterial blood gas samples were taken every 30 min, and ventilation was adjusted in such a way that proper blood gas levels were maintained, (i.e., Pa_{O_2} approximately 13.3 kPa and Pa_{CO_2} approximately 5.0 kPa). We maintained the pH at 7.40 by administering sodium hydrogen carbonate ($NaHCO_3$, 4.2%) when required.

A catheter was introduced into the carotid artery for blood sampling (blood gases, unlabeled free fatty acids, lactate and glucose levels) and arterial pressure measurements. For measurement of cardiac output and central body temperature, a pulmonary artery catheter was introduced into the external jugular vein. A thoracotomy was performed through the fifth left intercostal space, and the heart was suspended in a pericardial cradle. To prevent blood clotting, 200 IU/kg heparin were given intraveneously. The electrocardiogram and pressure curves were continuously monitored, and central body temperature was maintained between 37°C and 38°C with a water-heated pad. Normal saline was infused to match losses caused by respiration and blood sampling.

Experimental Protocols

After thoracotomy, the proximal left anterior descending coronary artery (LAD) was isolated and cannulated with an 18-gauge polyethylene cannula. Immediately (several seconds) after cannulation, the LAD area was perfused with arterial blood withdrawn from a catheter in a femoral artery by an ECB system (volume 18 mL). The LAD proximal to the site of cannulation was ligated, and a calibrated roller pump (Miniplus 3; Gilson, Middleton, WI) was used to adjust coronary flow. The perfusion pressure was measured at the proximal entrance of the cannula. The temperature of the blood was maintained at central body temperature by means of a heat exchanger (counter current principle). To obtain normal perfusion, we adjusted the perfusion pressure to match mean aortic pressure.

Three groups of animals were investigated (Fig. 2):

1. Control (n = 3). The LAD flow was maintained on a normal level to assess any effects of the ECB procedure on myocardial blood flow (MBF) and IFA kinetics and metabolism.
2. Ischemia (n = 9). The LAD flow was reduced by the ECB to one third of the individual control value (t = −30 min).
3. Hypoxia (n = 5). After equilibration of normal arterial blood flow, the LAD area was perfused by venous blood withdrawn from a femoral vein (t = −30 min). The transition from arterial to venous blood flow was instantaneous, whereas the flow of the roller pump remained unaltered.

In the control and ischemic groups, MBF was measured by the radiolabeled microsphere technique (*13*) 25 min after the LAD flow was adjusted (t = −5 min). In the hypoxic group, microspheres were injected during the period of arterial ECB perfusion

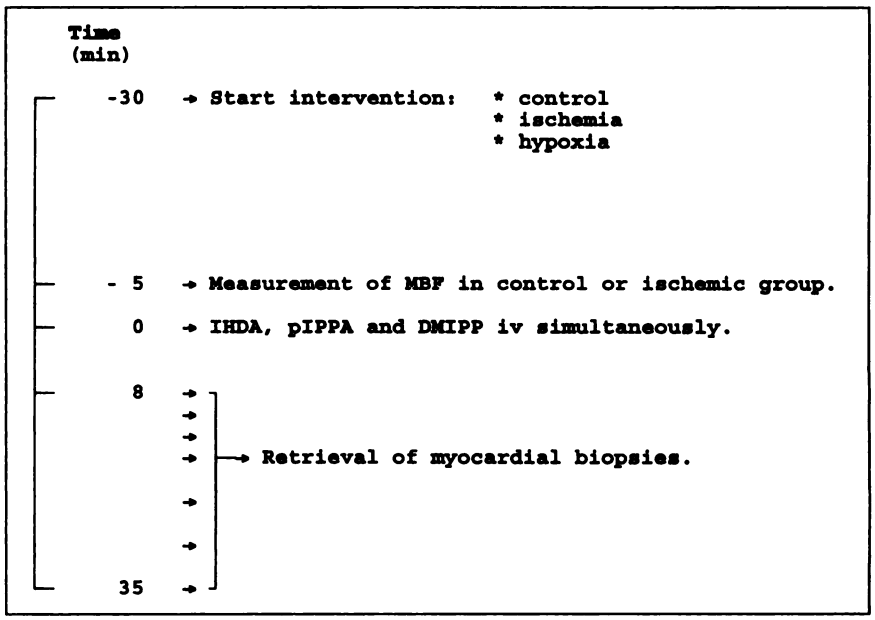

FIGURE 2. Time schedule of experimental protocols. Measurement of myocardial blood flow (MBF) in hypoxic group occurs just before intervention starts.

just before the transition to venous blood perfusion, which lasted another 30 min before the administration of the IFAs (t = 0). ^{46}Sc-labeled microspheres (diameter = 15 ± 1 μm; New England Nuclear Lifescience Products, Boston, MA) were suspended in normal saline and one drop of Tween-80 and were thoroughly mixed using a vortex shaker. Mixing was continued until the solution was injected through a cannula in the left atrium and approximately 3 million microspheres (0.1 mCi) were injected. Withdrawal of the arterial reference sample at the site of a carotid artery was initiated just before the injection of microspheres and was continued with a roller pump for 2 min at a rate of 18.2 mL/min.

Through a cephalic vein at t = 0 (30 min after introduction of the intervention), a mixture of 159.1 ± 14.8 MBq (4.3 ± 0.4 mCi) ^{123}I-IHDA (specific activity [SA] = 148 MBq/mg [4.0 mCi/mg]; Cygne, Eindhoven, The Netherlands), 81.4 ± 7.4 MBq (2.2 ± 0.2 mCi) ^{131}I-pIPPA (SA = 214.6 MBq/mg [5.8 mCi/mg]; Cygne) and 40.7 ± 3.7 MBq (1.1 ± 0.1 mCi) ^{125}I-DMIPP (SA = 296 MBq/mg [8.0 mCi/mg]; Oak Ridge National Laboratory, Oak Ridge, TN) was injected in one bolus. Thereafter, myocardial biopsy specimens, weighing 28.7 ± 9.3 mg, from both the normal (supplied by the left circumflex) and the ECB-perfused region were taken with a fast-spinning hollow needle at 8, 11, 15, 20, 25, 30 and 35 min after injection. Immediately after specimens were rinsed in ice-cold saline, they were frozen in liquid nitrogen. The time between taking the biopsy samples and freezing them was less than 20 s.

At t = 40 min, black India ink was administered into the ECB system to determine the ECB-perfused region; it was immediately followed by intracavitary administration of potassium chloride to induce cardiac arrest. After cardiac arrest, the heart was rapidly excised and was rinsed in ice-cold saline. The left ventricle was cut into five transverse slices of equal thickness, which were parallel to the atrial ventricular ring. The slices were cut into eight radial segments, each of which was divided into subepicardial, midcardial and subendocardial samples. The samples were classified as being entirely stained (control, ischemic or hypoxic myocardial region) or not stained at all (natively perfused myocardium). All small biopsy specimens taken for the assessment of IFA metabolism appeared retrospectively to be taken from the proper region. After weighing the myocardial samples (mean 1.038 ± 0.323 g) and blood reference samples, we counted the radioactivity for 5 min in the gamma well counter approximately 1 mo ($t_{1/2}$ ^{46}Sc = 84 d) after the experiment. From the weight and activity data, we calculated the myocardial blood flow (mL/g/min).

Counting Procedure and Biochemical Analysis

After weighing them, we submerged the biopsy specimens in vials filled with liquid nitrogen and counted them for 2 min in a gamma well counter (Compu Gamma 1282; LKB-Wallac, Turku, Finland) with window settings appropriate for ^{123}I, ^{125}I and ^{131}I. Background, decay and crossover were corrected for in all measurements.

Lipid extraction of biopsy specimens was performed using the method of Bligh and Dyer (*14*). Frozen biopsies were transferred to a Potter-Elvehjem glass–Teflon homogenizer. Ice-cold water (0.6 mL) was added, and the tissue was homogenized for 20 s at 0°C. Thereafter, 2.25 mL chloroform/methanol (1:2 by volume) were added, and homogenization was continued for 1 min at 0°C. The homogenate was transferred to a glass tube and the tissue residue remaining in the Potter tube was re-extracted twice with 1.0 mL chloroform/methanol/water (1:2:0.8 by volume). The two extracts were combined with the homogenate and were centrifuged for 5 min at 1500*g*. The supernatant was pipetted off and the remaining pellet was washed once by suspension in 1.0 mL chloroform/methanol/water (1:2:0.8 by volume). After centrifugation, this supernatant was added to the supernatant obtained earlier. Water (1.4 mL) and chloroform (1.4 mL) were added to the supernatant to initiate phase separation. After thorough mixing and centrifugation, the aqueous phase containing the water soluble oxidation products was separated from the chloroform phase containing the lipids. The chloroform fraction was dried under a stream of nitrogen (40°C). The three fractions (lipid phase, aqueous phase and pellet) were counted for 2 min in the gamma well counter.

Lipid fractions were redissolved in 70 μL chloroform/methanol (2:1 by volume). To analyze the neutral and polar lipid classes, we used two separate thin-layer chromotography (TLC) systems. Thirty microliter aliquots of the lipid extracts were spotted on both TLC plates, and standards were applied. After chromatography, the

plates were air dried, and individual lipids were found by exposing the plates to iodine vapor.

Neutral lipids were separated on plastic-backed silica gel TLC plates (Merck no. 5748; Merck, Darmstadt, Germany) using hexane/diethyl ether/acetic acid (65:35:1 by volume) as the developing solvent. The following fractions were obtained: polar lipids (R_f = 0.00), diacylglycerols (R_f = 0.17), fatty acids (R_f = 0.28), triacylglycerols (R_f = 0.35–0.67) and cholesteryl esters (R_f = 0.78). Because previous studies revealed almost negligible activity in the cholesteryl ester fraction, this fraction was not further analyzed.

Polar lipids were separated on glass-backed silica gel high-performance TLC plates (Merck no. 5642) with chloroform/hexane/methanol/acetic acid/water (48:28:16:12:1.2 by volume) as developing solvent. Individual polar lipids were recovered by scraping the silica gel from the glass-backed TLC plates. The following fractions were obtained: lysophosphatidylcholine (R_f = 0.04), acylcarnitine (R_f = 0.12), phosphatidylcholine (R_f = 0.21), phosphatidylinositol (R_f = 0.29), phosphatidylserine (R_f = 0.40), phosphatidylethanolamine (R_f = 0.55), diphosphatidylglycerol (R_f = 0.68) and neutral lipids ($R_f > 0.80$). The lipid fractions were counted for 5 min in the gamma well counter.

Data Analysis

Counting rates, already corrected for physical half-life, were also corrected for the efficiency of the gamma well counter for the different radioisotopes, the weight of the biopsy specimen and the radioactivity injected. In addition, correction for recovery was made for the lipid extraction and both TLCs. The average recovery of the lipid extraction was 96%, and the balance of recovery of the TLCs was 88%. Slight differences in sampling time of biopsy specimens were corrected by linear interpolation of the two adjacent sampling times. Data were expressed as disintegrations per minute per milligram tissue per millicurie injected fatty acid (dpm/mg/mCi) and were presented as time-activity curves. The relative distribution of the various fractions of total myocardial activity was calculated and expressed as a percentage (mean ± SD). Fractions containing radioactivity that failed to exceed twice the level of the background, which was the case in some polar lipid fractions, were considered negligible.

Clearance rates of total and aqueous phase activity were calculated by fitting the averaged data with a monoexponential curve and were expressed as half-time values (min). Spatial heterogeneity of MBF and fatty acid uptake in the heart (*15,16*) did not allow fitting time-activity curves for data from individual experiments. Finally, changes in metabolism due to ischemia or hypoxia were different between the IFAs. For comparison, these changes were indicated by the relative increase or decrease of the IFA at t = 8 min. The relative increase or decrease of IHDA, pIPPA or DMIPP activity is defined by the ratio of the difference between activities in ischemia (or hypoxia) versus normoxia relative to the activity in normoxia and expressed as a percentage.

For statistical analysis, Student *t* test for paired and unpaired data was applied when appropriate. $P < 0.05$ was considered significant.

RESULTS

Control Experiments

In control animals, mean aortic pressure (105 mm Hg at t = 0), cardiac output (4.6 L/min at t = 0) and heart rate (112 bpm at t = 0) were stable during the entire procedure. Total activity and the relative distribution over the various metabolic fractions for IHDA, pIPPA and DMIPP were similar in the native and ECB-perfused areas in three control experiments (data not given). Thus, within normal MBF values, the ECB technique did not alter the kinetics of the IFAs. We have demonstrated in our laboratory that the level of MBF can be adjusted properly and that IHDA metabolism and regional myocardial DMIPP activity are not altered by the ECB technique at normal MBF (*15,17*).

Hemodynamics and Substrate Levels

Intervention parameters, substrate levels and hemodynamic variables of the ischemic and hypoxic groups are listed in Table 1. In the ischemic group, the MBF in the ECB area was 0.50 ± 0.18 mL/g/min, which was significantly reduced (45% ± 15%, $P < 0.001$) compared with native perfused myocardium of 1.13 ± 0.25 mL/g/min. In the hypoxic group, the oxygen saturation of venous blood in the ECB-supplied area (75 ± 7 mm Hg) was significantly lower ($P < 0.001$) compared with oxygen saturation of arterial blood in the native perfused area (97 ± 1 mm Hg), whereas the MBF in both areas was similar.

Mean aortic pressure and cardiac output decreased significantly in both groups, suggesting a decline of myocardial mechanical performance. Plasma levels of glucose and lactate in ECB-supplied blood of both groups were in normal ranges and were similar to natively supplied blood.

Metabolism of Radioiodinated Fatty Acids in Ischemia and Hypoxia

Time-activity curves of IHDA, pIPPA and DMIPP in normoxic, ischemic and hypoxic myocardium are presented in Figure 3. Time-activity curves for normoxic, native perfused myocardium in the ischemic and hypoxic groups were similar (Tables 2, 3 and 4); therefore, only the curves for the normoxic myocardium of the ischemic group are presented for comparison.

Total Activity. In ischemic, but not in hypoxic myocardium, IHDA, pIPPA and DMIPP total activities at t = 8 min were significantly lower (all *P* values < 0.001) versus normal myocardium (Tables 2 and 3). The relative decrease of total activity in ischemia was not significantly different among IHDA (52% ± 14%), pIPPA (56% ± 15%) or DMIPP (48% ± 12%) (Fig. 4). The clearance rate of IHDA and pIPPA in ischemia and in hypoxia was prolonged versus normoxia (Table 4). In contrast, the clearance rate of DMIPP total activity in ischemia and hypoxia was reduced versus normoxia (Table 4).

Aqueous Phase. The aqueous phase activities of IHDA, pIPPA and DMIPP in ischemia and hypoxia were significantly lower than in normoxia (Tables 2 and 3). In ischemia and hypoxia, the relative decrease in aqueous phase activity was significantly higher ($P < 0.05$) for pIPPA versus IHDA (Fig. 4). The relative decrease of DMIPP aqueous phase activity was significantly lower versus IHDA and pIPPA (both *P* values < 0.001) in ischemia. The clearance rate of IHDA and pIPPA aqueous phase activity in ischemia and in

TABLE 1
Intervention Parameters, Plasma Substrate Levels and Hemodynamic Variables in Ischemic and Hypoxic Groups

	Area	Ischemia (n = 9)	*P* value vs. native	Hypoxia (n = 5)	*P* value vs. native
Intervention parameters					
MBF (mL/g/min)	Native	1.13 ± 0.25		1.28 ± 0.30	
	ECB	0.50 ± 0.18	0.001	1.31 ± 0.29	NS
Po_2 (mm Hg)	Native	104 ± 6		102 ± 3	
	ECB	102 ± 5	NS	46 ± 5	0.001
O_2 saturation (%)	Native	98 ± 1		97 ± 1	
	ECB	98 ± 1	NS	75 ± 7	0.001
Substrates					
Fatty acids (mmol/L)	Native	0.89 ± 0.33		0.86 ± 0.50	
	ECB	0.88 ± 0.29	NS	0.93 ± 0.52	NS
Glucose (mmol/L)	Native	7.5 ± 1.5		7.4 ± 0.5	
	ECB	7.5 ± 1.4	NS	7.2 ± 0.6	NS
Lactate (mmol/L)	Native	1.9 ± 1.4		1.7 ± 0.9	
	ECB	1.9 ± 1.3	NS	1.8 ± 0.8	NS
	Time (min)		*P* value vs. t = −30		*P* value vs. t = −30
Hemodynamics					
MAP (mm Hg)	−30	109 ± 10		106 ± 5	
	0	102 ± 10	0.05	99 ± 4	0.05
	30	91 ± 11	0.001	88 ± 5	0.001
Heart rate (bpm)	−30	106 ± 23		134 ± 24	
	0	138 ± 28	0.001	147 ± 23	0.05
	30	150 ± 21	0.001	150 ± 23	0.001
CO (L/min)	−30	4.7 ± 0.9		4.8 ± 1.4	
	0	4.3 ± 0.7	0.001	4.0 ± 1.1	0.001
	30	4.0 ± 0.9	0.001	3.8 ± 1.2	0.001

MBF = myocardial blood flow; ECB = extracorporeal bypass; NS = not significant; MAP = mean arterial pressure; bpm = beats per minute; CO = cardiac output.
At t = −30 min, intervention, ischemia or hypoxia was started; at t = 0 min, radioiodinated fatty acid analogs were injected intravenously.

hypoxia was prolonged versus normoxia. The half-time value of DMIPP aqueous phase activity was lower in ischemia and hypoxia than in normoxia (Table 4).

Unmetabolized Radioiodinated Fatty Acids. In ischemia, the levels of unmetabolized IHDA, pIPPA and DMIPP at t = 8 min were significantly elevated compared with normoxia (Table 2).

Diacylglycerols. In normoxia, low levels of IHDA, pIPPA or DMIPP activity were found in diacylglycerols, which did not change significantly in ischemia or hypoxia (Tables 2 and 3).

Triacylglycerols. Incorporation of IHDA and pIPPA into triacylglycerols was significantly higher in both ischemia and hypoxia versus normoxia (Tables 2 and 3). Incorporation of DMIPP into triacylglycerols was also significantly higher in ischemia at t = 35 min (Table 2) and in hypoxia (Table 3), both versus normoxia.

Polar Lipids and Polar Lipid Fractions. Incorporation of IHDA and pIPPA into polar lipids at t = 8 min was significantly higher in both ischemia and hypoxia versus normoxia (Table 2 and 3). Incorporation of DMIPP into polar lipids was low and did not change significantly in ischemia or hypoxia versus normoxia (Tables 2 and 3).

Incorporation of IHDA, pIPPA and DMIPP into polar lipid fractions in normoxic myocardium has been described previously (*18*). During ischemia and hypoxia, activity of pIPPA and IHDA in acylcarnitine increased significantly (Tables 2 and 3). In normal myocardium, IHDA and pIPPA are substantially incorporated into phosphatidylcholine, and this pattern did not change significantly in ischemia or hypoxia. IHDA is predominantly incorporated into phosphatidylinositol under normal conditions. The relative incorporation of IHDA into this phospholipid decreased in ischemia ($P < 0.05$) and in hypoxia (not significant). DMIPP is mainly incorporated into phosphatidylserine, which decreased significantly in ischemia (Table 2) but not in hypoxia.

DISCUSSION

To be useful for fatty acid scintigraphy, IFAs should be recognized by the cardiomyocytes as a natural long-chain fatty acid. Therefore uptake, oxidation or lipid incorporation of IFAs should resemble the kinetics of natural fatty acids. Further, parameters obtained by external scintigraphy (total activity, clearance rate) should provide information on discrete aspects of fatty acid metabolism, such as uptake, oxidation or lipid incorporation. When IFAs meet these criteria, IFA scintigraphy could be useful for diagnostic purposes, such as detection of ischemic heart disease or

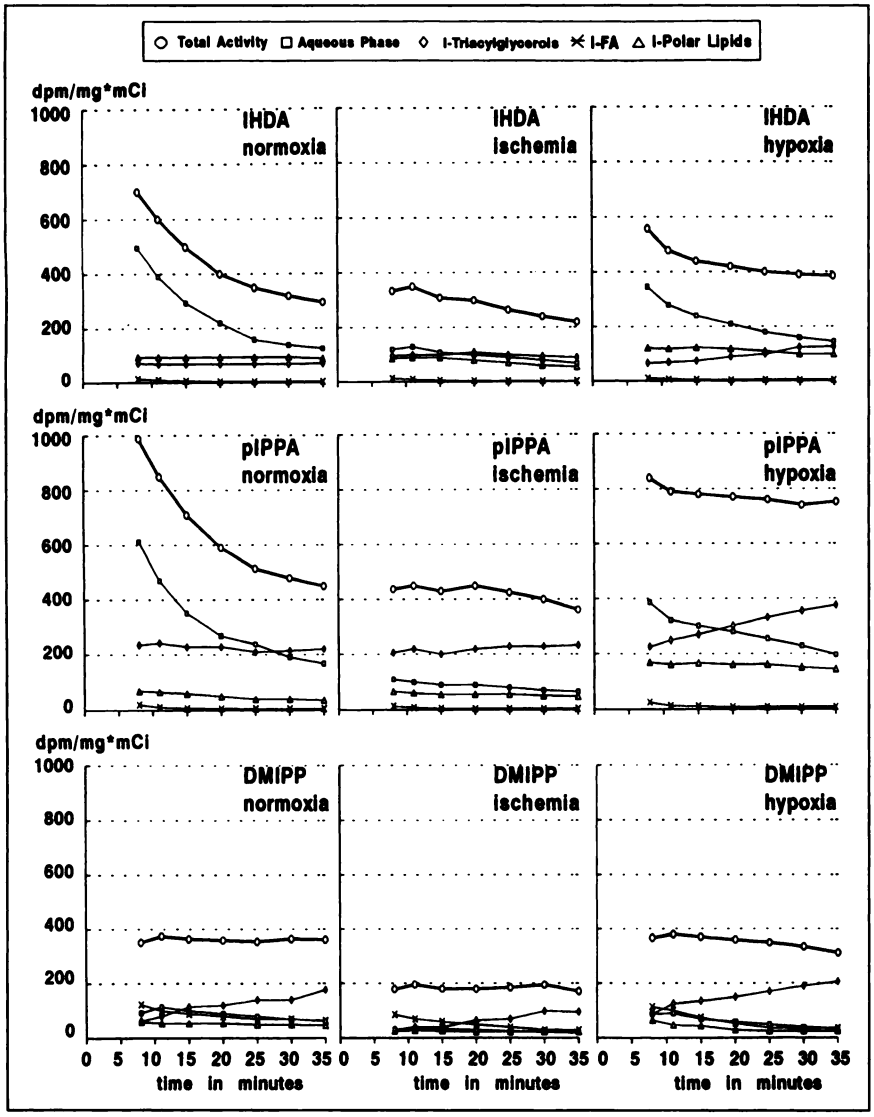

FIGURE 3. Time-activity curves of averaged data (dpm/mCi/mg) of total activity and major fractions in normoxic (left panels, n = 9), ischemic (middle panels, n = 9) and hypoxic (right panels, n = 5) canine myocardium after simultaneous intravenous administration of IHDA, pIPPA and DMIPP.

assessment of myocardial viability. Therefore, the myocardial metabolism of key examples of different iodinated fatty acid classes was studied under ischemic conditions to evaluate the relative merits or drawbacks for fatty acid scintigraphy.

In ischemia, we found that peak total activity and the oxidation fraction of IHDA, pIPPA and DMIPP decreased, whereas incorporation into triacylglycerols relatively increased. These alterations in kinetics of the three IFAs resemble the ischemic changes described for natural long-chain fatty acids (*2–4*). The decreases in peak total activity of IHDA, pIPPA and DMIPP were not different, suggesting a similar mechanism of uptake. Levels of unmetabolized IHDA, pIPPA and DMIPP and intermediates of IHDA and pIPPA in the acylcarnitine fraction were elevated in ischemia, resembling ischemic alterations of natural fatty acid shortly after cellular uptake and before mitochondrial oxidation (*3*).

The dimethyl-substituted DMIPP showed a different behavior compared with IHDA and pIPPA. In normoxia, DMIPP was retained in the myocardium and was mainly incorporated into triacylglycerols. This is consistent with our earlier studies, which showed that DMIPP is a poor oxidizable substrate for the normoxic myocardium but is readily stored in the neutral lipid pool (*10,19*). In contrast to IHDA and pIPPA, relatively high levels of unmetabolized DMIPP were found in normoxia, and levels were significantly raised in ischemia, suggesting a relatively lower activation rate (conversion to the corresponding acyl-coenzyme-A derivate) of this dimethyl analog. At the end of the experiment, levels of unmetabolized DMIPP were again comparable in normoxia versus ischemia, which may be partly explained by backdiffusion of the fatty acid from tissue to the blood compartment, which is relatively higher in ischemia than in normoxia (*20,21*).

Although the kinetics of the straight-chain IHDA and pIPPA show major similarities, some differences between these two iodinated fatty acids should be acknowledged. The radioactivity in the aqueous phase fraction was relatively higher for IHDA compared with pIPPA in normoxia and the

TABLE 2
Total Activity and Relative Distribution of Metabolites in Normoxic and Ischemic Myocardium of Dogs (n = 9) in Ischemic Group

	t = 8 min			t = 35 min		
	Normoxia	*P*	Ischemia	Normoxia	*P*	Ischemia
Total activity (dpm/mg/mCi)						
IHDA	700 ± 267	0.001	335 ± 158	296 ± 105	0.05	219 ± 112
pIPPA	988 ± 318	0.001	438 ± 180	450 ± 152	0.05	360 ± 134
DMIPP	352 ± 146	0.001	179 ± 82	362 ± 157	0.001	171 ± 75
Relative distribution (%)						
Aqueous phase						
IHDA	71 ± 9	0.001	36 ± 9	43 ± 11	0.05	31 ± 11
pIPPA	62 ± 10	0.001	25 ± 8	37 ± 17	0.001	18 ± 8
DMIPP	26 ± 11	0.05	18 ± 3	17 ± 5	NS	11 ± 4
Triacylglycerols						
IHDA	11 ± 6	0.001	29 ± 9	24 ± 15	0.001	41 ± 17
pIPPA	24 ± 11	0.001	47 ± 14	49 ± 18	0.001	65 ± 10
DMIPP	18 ± 7	NS	16 ± 6	48 ± 8	0.05	56 ± 7
Unmetabolized IFA						
IHDA	2 ± 1	0.05	4 ± 2	1 ± 1	NS	1 ± 1
pIPPA	2 ± 1	0.05	3 ± 1	1 ± 0	NS	1 ± 0
DMIPP	35 ± 13	0.05	48 ± 9	18 ± 9	NS	17 ± 10
Diacylglycerols						
IHDA	1 ± 1	NS	2 ± 2	1 ± 1	NS	1 ± 0
pIPPA	3 ± 1	NS	4 ± 2	2 ± 1	NS	2 ± 1
DMIPP	2 ± 1	NS	2 ± 1	1 ± 0	NS	2 ± 1
Polar lipids						
IHDA	13 ± 6	0.05	26 ± 5	30 ± 9	NS	25 ± 10
pIPPA	7 ± 1	0.05	15 ± 3	8 ± 2	0.05	13 ± 2
DMIPP	17 ± 5	NS	14 ± 4	13 ± 6	NS	13 ± 5
Polar lipid fractions						
LPC						
IHDA	—		—	—		—
pIPPA	13 ± 8	NS	8 ± 6	—		—
DMIPP	—		—	—		—
AC						
IHDA	9 ± 5	0.001	26 ± 11	—		—
pIPPA	14 ± 7	0.001	36 ± 14	8 ± 5	0.05	13 ± 9
DMIPP	—		—	—		—
PC						
IHDA	24 ± 12	NS	22 ± 11	31 ± 12	NS	26 ± 12
pIPPA	35 ± 12	NS	33 ± 16	39 ± 10	0.05	52 ± 22
DMIPP	—		—	—		—
PI						
IHDA	35 ± 19	0.05	24 ± 17	49 ± 25	NS	45 ± 27
pIPPA	—		—	—		—
DMIPP	—		—	—		—
PS						
IHDA	8 ± 7	NS	5 ± 4	5 ± 4	NS	5 ± 5
pIPPA	10 ± 8	NS	9 ± 7	21 ± 7	0.05	8 ± 6
DMIPP	31 ± 24	0.05	11 ± 7	23 ± 10	NS	16 ± 6
PE						
IHDA	13 ± 11	NS	12 ± 10	14 ± 8	NS	18 ± 14
pIPPA	10 ± 8	NS	6 ± 7	14 ± 5	NS	6 ± 5
DMIPP	—		—	—		—

IHDA = 17-iodoheptadecanoic acid; pIPPA = 15-(p-iodophenyl)pentadecanoic acid; DMIPP = 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid; IFA = radioiodinated fatty acid; LPC = lysophophatidylcholine; AC = acylcarnitine; PC = phosphatidylcholine; PI = phosphatidylinositol; PS = phosphatidylserine; PE = phosphatidylethanolamine.

Relative distribution of metabolites is expressed as percentage of total activity in biopsy specimen and for polar lipid fractions as percentage of total polar lipids. Values of normoxia versus ischemia were analyzed by paired Student *t* test and expressed as *P* value (NS = not significant). Dashes indicate polar lipid fractions that were below detection limit (see Materials and Methods section for criteria).

TABLE 3
Total Activity and Relative Distribution of Metabolites in Normoxic and Hypoxic Myocardium of Dogs (n = 5) in Hypoxic Group

	t = 8 min			t = 35 min		
	Normoxia	*P*	Hypoxia	Normoxia	*P*	Hypoxia
Total activity (dpm/mg/mCi)						
IHDA	627 ± 212	NS	558 ± 197	263 ± 40	0.05	385 ± 191
pIPPA	923 ± 323	NS	840 ± 289	432 ± 112	0.05	752 ± 412
DMIPP	345 ± 165	NS	367 ± 218	358 ± 137	NS	312 ± 152
Relative distribution (%)						
Aqueous phase						
IHDA	76 ± 8	0.05	62 ± 8	51 ± 10	0.05	38 ± 9
pIPPA	66 ± 8	0.05	46 ± 10	45 ± 15	0.05	26 ± 12
DMIPP	32 ± 6	0.05	24 ± 4	21 ± 11	0.05	11 ± 3
Triacylglycerols						
IHDA	9 ± 6	0.05	12 ± 5	21 ± 12	0.05	33 ± 11
pIPPA	20 ± 8	0.05	27 ± 8	42 ± 15	0.05	50 ± 10
DMIPP	19 ± 15	0.05	24 ± 11	51 ± 18	0.05	66 ± 10
Unmetabolized IFA						
IHDA	2 ± 2	NS	2 ± 1	1 ± 0	NS	1 ± 1
pIPPA	2 ± 2	NS	3 ± 1	1 ± 0	NS	1 ± 1
DMIPP	29 ± 14	NS	31 ± 8	12 ± 8	NS	12 ± 6
Diacylglycerols						
IHDA	1 ± 1	NS	1 ± 1	1 ± 0	NS	1 ± 1
pIPPA	2 ± 1	NS	3 ± 1	2 ± 0	NS	3 ± 1
DMIPP	1 ± 0	NS	1 ± 1	1 ± 0	NS	1 ± 0
Polar lipids						
IHDA	11 ± 2	0.05	22 ± 5	25 ± 4	NS	26 ± 5
pIPPA	8 ± 2	0.05	20 ± 8	9 ± 3	0.05	19 ± 19
DMIPP	17 ± 4	NS	18 ± 5	13 ± 1	NS	9 ± 5
Polar lipid fractions						
LPC						
IHDA	—		—	—		—
pIPPA	11 ± 8	NS	9 ± 5	—		—
DMIPP	—		—	—		—
AC						
IHDA	7 ± 4	0.05	19 ± 10	—		—
pIPPA	18 ± 5	0.05	29 ± 13	12 ± 7	0.05	25 ± 22
DMIPP	—		—	—		—
PC						
IHDA	30 ± 2	NS	28 ± 16	31 ± 9	NS	37 ± 14
pIPPA	34 ± 6	NS	41 ± 32	37 ± 9	0.05	53 ± 39
DMIPP	—		—	—		—
PI						
IHDA	41 ± 21	NS	34 ± 18	44 ± 16	NS	38 ± 16
pIPPA	—		—	—		—
DMIPP	—		—	—		—
PS						
IHDA	7 ± 3		—	5 ± 1		—
pIPPA	9 ± 5		—	20 ± 5		—
DMIPP	44 ± 24	NS	45 ± 42	27 ± 14	NS	21 ± 17
PE						
IHDA	14 ± 4	NS	9 ± 4	16 ± 4	NS	15 ± 5
pIPPA	8 ± 3	NS	5 ± 3	12 ± 3	NS	5 ± 5
DMIPP	—		—	—		—

IHDA = 17-iodoheptadecanoic acid; pIPPA = 15-(p-iodophenyl)pentadecanoic acid; DMIPP = 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid; IFA = radioiodinated fatty acid; LPC = lysophophatidylcholine; AC = acylcarnitine; PC = phosphatidylcholine; PI = phosphatidylinositol; PS = phosphatidylserine; PE = phosphatidylethanolamine.

Relative distribution of metabolites is expressed as percentage of total activity in biopsy specimen and for polar lipid fractions as percentage of total polar lipids. Values of normoxia versus ischemia were analyzed by paired Student *t* test and expressed as *P* value (NS = not significant). Dashes indicate polar lipid fractions that were below detection limit (see Materials and Methods section for criteria).

TABLE 4
Clearance Rates of Total Activity and Aqueous Phase Fraction in Ischemic and Hypoxic Groups

	Ischemic group		Hypoxic group	
	Normoxia	Ischemia	Normoxia	Hypoxia
Total activity				
IHDA	22	44	22	50
pIPPA	24	95	25	169
DMIPP	528	409	483	115
Aqueous phase				
IHDA	14	33	15	22
pIPPA	14	36	16	27
DMIPP	47	35	49	20

IHDA = 17-iodoheptadecanoic acid; pIPPA = 15-(p-iodophenyl) pentadecanoic acid; DMIPP = 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid.

Time-activity curves of averaged data (ischemic group; n = 9; hypoxic group; n = 5) were fitted with monoexponential curve. Clearance rates are expressed as half-time values in minutes.

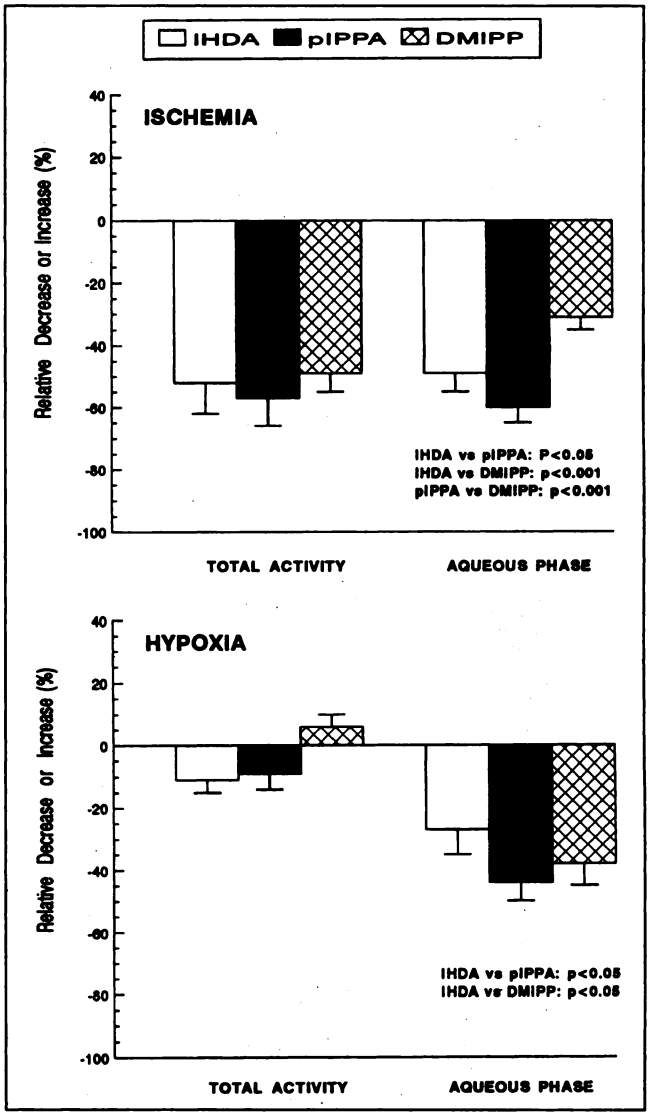

FIGURE 4. Changes in metabolism due to ischemia (top) or hypoxia (bottom) were different between IHDA, pIPPA and DMIPP. The changes were indicated by relative decrease or increase at t = 8 min. Relative decrease or increase of IHDA, pIPPA or DMIPP activity is ratio of difference between activities in ischemia (or hypoxia) versus normoxia relative to activity in normoxia.

relative decrease was significantly lower for IHDA compared with pIPPA. In addition, the increase in total activity half-time values during ischemia or hypoxia was higher for pIPPA than for IHDA. A possible explanation for this difference in oxidation rate could be that pIPPA is less easily transported into the mitochondria to be oxidized; instead, a relatively larger fraction is incorporated into triacylglycerols, probably due to the unphysiological phenyl-substituted structure of the fatty acid analog. This suggestion is supported by the higher acylcarnitine fractions for pIPPA compared with IHDA, not only in normoxia but also even more pronounced during ischemia and hypoxia. Another explanation might be nonspecific (i.e., not oxidation related) deiodination of IHDA or intermediates. Nonspecific deiodination of the intact IHDA chain has been suggested previously (*7*), but this has not been proven (*22*). However, nonspecific deiodination of some of the short-chain intermediates of IHDA, the concentrations of which may rise as a result of incomplete oxidation during oxygen restriction (*23*), may occur, leading to a certain amount of nonoxidation-related activity of free iodine in the aqueous phase. In case of intermediates of pIPPA, the iodophenyl bond will prevent this nonspecific deiodination (*11*).

In the aqueous phase in normoxia, there was a considerable amount of DMIPP activity that was significantly lower during ischemia and hypoxia, suggesting the presence of an oxidation-related intermediate in the myocardium. As suggested by Knapp et al. (*10*), an α-hydroxy metabolite might correlate with this finding. Moreover, the relative mobility of α-OH-BMIPP, the monomethylated analog, is comparable with para-iodobenzoic acid (*12*), which is found in the aqueous fraction in the chemical analysis of this study. Furthermore, levels of DMIPP into the acylcarnitine pool remained below the detection limit, suggesting that the DMIPP derivates are poor substrates for carnitine acyltransferase, which might be the cause of cardiac inability to oxidize DMIPP at a physiologically significant rate.

The incorporation of IHDA and pIPPA into the polar lipid fraction showed a relative increase during ischemia and hypoxia, a finding consistent with Rosamund et al. (*4*), who studied 1-^{11}C and 1-^{14}C-palmitate in dogs with regional ischemia by ECB perfusion and found that the fraction of activity in the polar lipid fraction doubled in ischemia compared with normoxia. The polar lipid fraction is predominantly represented by phospholipids, yet the increase of the acylcarnitine subfraction (which is a polar lipid but is not a phospholipid) is mainly responsible for the increase in the polar lipid fraction during oxygen deprivation. In contrast, the total phospholipid pool has been reported to decrease,

mainly the phosphatidylcholine and phosphatidylethanolamine subfractions, as indicated by the release of arachidonic acid during ischemia (*3,24*). In this study, incorporation of IHDA and pIPPA into the phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine subfractions showed a small decrease during oxygen deprivation.

During hypoxia, aqueous phase fractions of the three IFAs studied decreased and incorporation into triacylglycerols increased. Thus, oxygen deprivation decreases the catabolism and increases esterification of the IFAs independent of myocardial blood flow. In the hypoxic experiments, total activity of the IFAs at 8 min remained unchanged, suggesting that the uptake of the IFAs is related to flow. However, it is well known that other parameters like plasma substrate levels, tissue energy state and acidity also determine fatty acid uptake (*3*). In acute and in chronic ischemia, uncoupling of fatty acid uptake and flow are reported. Increased as well as decreased fatty acid uptake relative to flow have been reported previously (*5,15,25–29*). Despite the reduction in oxygen supply in the hypoxic experiments, peak total activity remained unaltered; therefore, we may assume that the other determinants of fatty acid uptake mentioned previously were not disturbed.

The hypoxic experiments also confirmed the enhanced handling of IHDA, pIPPA and DMIPP by nonoxidative pathways, especially incorporation into the triacylglycerol pool, which is consistent with metabolism of natural fatty acids during oxygen deprivation (*2–4*).

For the IFAs studied, the clearance rates of total activity and various fractions in normoxic myocardium are in line with our previous studies (*19*). In ischemia, clearance of total activity of IHDA and pIPPA decreased, which is in line with studies using 1-^{11}C-palmitate (*4,30–31*). Also, in hypoxia, clearance of total activity of IHDA and pIPPA was delayed, suggesting that delayed clearance is related primarily to decreased oxidative metabolism rather than to alteration in coronary blood flow. Lerch et al. (*30*) studied 1-^{11}C-palmitate in ischemic and hypoxic conditions and found a similar pattern. The relatively enhanced incorporation during ischemia or hypoxia into lipid pools with a slow turnover rate is another factor contributing to the delayed clearance of IHDA and pIPPA total activity.

The relatively higher clearance rate of DMIPP observed during ischemia or hypoxia compared with normoxia may be explained by clearance of unmetabolized DMIPP and aqueous phase activity, probably α-OH-DMIPP (vide supra).

Implications for In Vivo Scintigraphy

In vivo scintigraphy identifies total myocardial activity. Therefore, parameters such as peak activity, isotope content and clearance rates can be deduced and these parameters reflect only some parts of fatty acid metabolism. However, the findings in this study underscore the notion that fatty acid metabolism and alterations during ischemia are complex at the tissue level.

Total activity of IHDA, pIPPA and DMIPP consistently decreased in experimental ischemia, therefore, decreased uptake of these three IFAs in scintigraphy most likely indicates myocardial ischemia in patients. Clearance of IHDA and pIPPA is delayed in ischemic and hypoxic myocardium, therefore, this parameter has been suggested as a sensitive marker for detection of coronary artery disease in patients (*32–34*). However, in some studies, no significant differences between normal and ischemic regions were found (*35*). Methodological differences, like the duration of acquisition and background correction procedures, are most likely responsible for the differences in outcome of these studies (*34*).

Unfortunately, the rapid clearance of IHDA and pIPPA in normal myocardium hampers proper SPECT imaging; whereas in planar scintigraphy, overprojection of normal and ischemic myocardium may easily occur. Another problem for IHDA and pIPPA scintigraphy in humans is that no satisfactory method has been found to correct for background activity from the oxidation product, which originates not only from the heart but from various other organs, such as the liver and muscles, where fatty acids are metabolized.

Because total myocardial activity of DMIPP does not show significant clearance, it may be suitable for SPECT acquisitions to study regional fatty acid uptake. Moreover, because the carbon chain of DMIPP is not catabolized, background activity remains at a low level (*19*), resulting in high-quality SPECT images (*36*). Our data suggest that DMIPP activity may provide information on fatty acid uptake and triacylglycerol incorporation. However, direct information on mitochondrial β-oxidation cannot be provided because our analysis strongly suggests that mitochondrial utilization of DMIPP does not significantly contribute to myocardial DMIPP metabolism.

Methodological Considerations

We have not investigated natural fatty acids, like ^{11}C- or ^{14}C-labeled palmitate, because simultaneous measurements of radioactivity of ^{11}C or ^{14}C and the radioiodines in the same biopsy specimens and all chemical fractions could not be conducted. Therefore, only a qualitative comparison could be made between these iodinated fatty acid analogs and physiological fatty acids that are extensively documented in literature. Direct comparisons between a single IFA and palmitate have been performed previously, and major similarities have been observed (*37–39*).

The experimental setup proved to be hemodynamically stable, which is also indicated by the high number of successful experiments. This is, however, accomplished only when the biopsy specimens are small, which may lead to a high SD in the collected counts. This effect is caused primarily by the physiological, spatial heterogeneity of myocardial blood flow and metabolism and is not caused by measurement errors or other factors (*15*). The coefficient of variation, an indicator of heterogeneity, at 8 min was 0.38 for IHDA, 0.32 for pIPPA and 0.41 for DMIPP. Heterogeneity for DMIPP in 1-g samples was assessed at 0.13, and heterogeneity increases strongly when sample size decreases

(*16*). However, the relative distribution of the main fractions showed relatively small SDs in normoxia, suggesting that metabolic handling after uptake is rather homogeneous in normal myocardium.

In some polar lipid fractions, counting rates were sometimes low; therefore, we defined a detection limit (see Materials and Methods section) to leave out inaccurate measurements. Counting rates in some polar lipid fractions were low for various reasons, such as low incorporation (DMIPP), time elapsed (^{123}I) or the recovery of the silica gel plates. Therefore, the relative distribution of the polar lipid fractions is indicative rather than quantitative. In contrast, counting rates of total activity in almost all biopsy specimens were greater than 10,000, giving a very low relative measurement error of $<1\%$. Thus, these measurements were accurate.

CONCLUSION

Our findings show that the kinetics of IFAs are altered during oxygen deprivation in a similar fashion to the kinetics of natural fatty acids. However, the changes are different among IHDA, pIPPA and DMIPP, suggesting different metabolic handling and reflecting different aspects of myocardial fatty acid metabolism.

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