# 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

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The kinetics of 17-[$^{123}$I]iodoheptadecanoic acid (IHDA), 15-(p-[$^{125}$I]iodophenyl)pentadecanoic acid (pIPPA) and 15-(p-[$^{131}$I]iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPPA) were investigated in normal canine myocardium. After simultaneous intravenous injection, myocardial biopsy specimens and samples of arterial blood were taken over 80 min. IHDA showed the highest myocardial uptake (995 ± 248 dpm/mg·mCi versus pIPPA: 785 ± 197 dpm/mg·mCi, ns) and the largest size of oxidation (74% ± 4% versus pIPPA: 65% ± 5%, $p < 0.05$). Myocardial activity of IHDA decreased with a half-time value of 11.2 min (pIPPA: 13.2 min). Phospholipids were the main lipid fraction into which IHDA was incorporated, whereas pIPPA was predominantly incorporated into triacylglycerols. DMIPPA myocardial activity remained constant during the assay period and instead of being oxidized, DMIPPA was mainly incorporated into triacylglycerols (55% ± 12%). The myocardium-to-blood ratios of DMIPPA were greater than 10:1. The ratios at peak for IHDA and pIPPA were 4.1:1 and 3.9:1, respectively (both $p < 0.0001$ versus DMIPPA). In conclusion, differences have been found in the myocardial uptake, oxidation and lipid distribution of IHDA, pIPPA and DMIPPA. DMIPPA is a promising tracer for fatty acid uptake studies with single-photon emission computerized tomography because of its prolonged retention and high myocardium-to-blood ratios.

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The striking metabolic differences between normal and flow-deprived areas of the myocardium make metabolic tracers suitable tools to differentiate between normoxic and ischemic myocardial tissue. Since free fatty acids are an important energy source for normal myocardium (*1*), various radiolabeled free fatty acids have been developed to study myocardial metabolism noninvasively, which include radioiodinated fatty acids (IFAs). Because iodine-123 has favorable physical properties (energy: 159 keV, physical half-life: 13.2 hr), conventional gamma cameras can be used.

An aliphatic fatty acid radiolabeled with iodine in the omega position, 17-iodoheptadecanoic acid (IHDA), is avidly taken up in normal myocardium and oxidized with concomitant release of the radioiodine (Fig. 1). The free iodide leaves the cell and enters the blood, resulting in low heart-to-blood ratios. Although unspecific metabolic deiodination of IHDA has also been suggested (*2*), it has been demonstrated that release of radioiodine results from beta oxidation (*3,4*). Because of the high background activity of IHDA, Machulla et al. (*5*) developed 15-(p-iodophenyl)-pentadecanoic acid (pIPPA, Fig. 1). The pIPPA analog is catabolized to para-iodobenzoic acid and eliminated by the kidneys and the liver (*6,7*). This would lower the levels of radioactivity in the blood, resulting, as is suggested, in a favorable target-to-background ratio.

The rapid myocardial clearance of both IHDA and pIPPA limits their use for assessment of regional myocardial uptake by single-photon emission computerized tomography (SPECT). The 3-monomethyl-substituted analog, 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPPA), exhibits myocardial clearance slow enough to permit regional distribution studies by SPECT. However, considerable myocardial clearance still exists (*8*), possibly due to catabolism by α-hydroxylation with sub-

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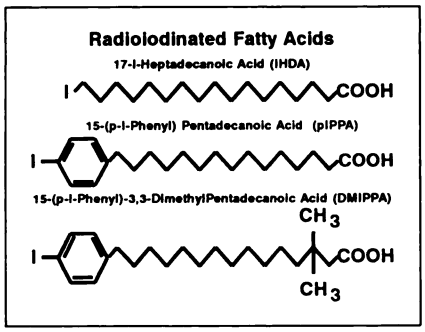


**FIGURE 1.** Structural formula of radioiodinated fatty acids.

sequent oxidation (*9,10*). To completely block catabolism, Knapp et al. (*9*) designed the "molecular microsphere": 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPPA, Fig. 1).

Since a direct comparison of the kinetics of IHDA, pIPPA and DMIPPA in the same animals has not yet been made, we have studied their uptake and metabolic characteristics in the normoxic heart after simultaneous administration of the three IFAs. Because the analysis of the time-activity curves in patients provides only a limited number of variables (peak activity, myocardial clearance and a relative size of oxidation), we focused on uptake, clearance rate, oxidation and incorporation into endogenous lipid pools. In this manner, we attempted to assess the relative merits of the three IFAs for the characterization of specific metabolic pathways. Finally, we calculated myocardium-to-blood ratios, an important factor in the quality of scintigraphic imaging.

## MATERIALS AND METHODS

### Radiolabeled Free Fatty Acids

Iodine-123-IHDA and $^{125}$I-pIPPA were obtained commercially from Cygne, (Eindhoven, The Netherlands) and had a specific activity of 4.0 mCi/mg and 5.6 mCi/mg, respectively. Iodine-131-DMIPPA was prepared by the thallation-iodination route (*9,11*) and had a specific activity of 8.0 mCi/mg. The fatty acids were complexed to 20% human serum albumin prior to administration (*9*).

### Animal Experiments

Six mongrel dogs (22–37 kg) were studied after overnight fasting. After intramuscular premedication with methadon (10 mg) and droperidol (15 mg), anesthesia was induced by thiopental (25 mg/kg) and maintained with droperidol (0.5 mg/kg·hr) and methadon (0.3 mg/kg/hr) in combination with $N_2O/O_2$ (2:1, by volume). The dogs were ventilated in such a way that proper blood gasses were maintained, i.e., $pO_2$ about 13.3 kPa and $pCO_2$ about 5.0 kPa and pH was kept at 7.4 by administering sodium hydrogen carbonate ($NaHCO_3$, 4.2%) when required. To prevent arrhythmias during biopsy retrieval, lidocaine (1.2 mg/kg·hr) was administered intravenously. A catheter was introduced into the carotid artery for blood sampling and pressure measurements. Cardiac output and central body temperature were measured with a Swan Ganz catheter. A thoracotomy was performed through the fifth left intercostal space and the heart was suspended in a pericardial cradle. The heart was paced at a frequency of 150 beats per min by an electrode attached to the left atrial appendage. The electrocardiogram and pressure curves were continuously monitored. Central body temperature was maintained between 37 and 38°C using a water heated pad beneath the dog.

### Biopsies and Blood Sampling

Via a cephalic vein, 3.8–6.4 mCi $^{123}$I-IHDA, 1.4–2.5 mCi $^{125}$I-pIPPA and 0.5–2.1 mCi $^{131}$I-DMIPPA were injected simultaneously in one bolus. Thirteen transmural myocardial biopsy specimens (weighing 52.9 ± 20.0 mg) were taken with a fast spinning hollow needle at 4.5, 5, 6, 8, 10, 12, 16, 20, 30, 45, 60, 70 and 80 min after administration of the IFAs. Immediately after rinsing in ice-cold saline, the biopsy specimens were frozen in liquid nitrogen. The time between taking the biopsy specimens and freezing required less than 20 sec. Arterial blood samples of 2 ml were taken at similar time intervals and stored on melting ice. Blood samples were drawn for analysis of arterial unlabeled fatty acid, lactate and glucose levels before (t = 0), at 30 and 60 min and at the end of the experiment.

### Chemical Analysis and Counting Procedure

*Biopsy Specimens.* After weighing, the biopsies were submerged in vials filled with liquid nitrogen and counted for 1 min in a gamma well counter (LKB-Wallac 1282 Compu Gamma, multi-isotope option) with window settings for $^{125}$I, $^{123}$I and $^{131}$I. Decay and crossover were corrected. The biopsy specimens were then ground with a glass pestle in a glass tube at 0°C and extracted using the Folch method (*12*). After centrifugation, the organic phase, the aqueous phase, which contained the oxidation products, and the pellet were separated. The organic phase was dried under a stream of nitrogen. The fractions were counted for 1 min in the gamma well counter. The dried organic phase, containing the lipids, was then redissolved in 100 μl of chloroform and applied to a Merck Alufolien 60 F254 chromatography plate which was developed three times using hexane/diethyl ether/acetic acid (80:20:1, by volume). Reference samples of lipids were also applied. After spraying with dichlorofluorescein, the plates were cut into appropriate strips containing phospholipids, diacylglycerols, fatty acids, triacylglycerols or cholesterol esters. Individual strips were counted for 5 min and corrected for crossover and decay.

*Blood Samples.* Aliquots (200 μl) were pipetted off and counted in the gamma well counter after correcting for crossover and decay. After centrifugation, 100 μl of plasma was pipetted off and counted. Lipid extraction and counting was performed as described for the biopsy specimens.

### Data Analysis

Biopsy and blood data of the labeled fatty acids were expressed as disintegrations per minute per milligram tissue per millicurie injected fatty acid (dpm/mg·mCi). Disintegrations per minute were calculated from the corrected count rates and the efficiency of the gamma well counter for the three radioisotopes separately. Corrections were made for loss of activity during the chemical work-up (recovery) and the count rates of the aqueous phase. Lipid fraction and pellet were summed and normalized to the total activity. The same procedure was performed for blood plasma. Slight differences in sampling time of biopsy specimens and blood samples were corrected by linear interpolation of the two adjacent sampling times. The relative distribution of all fractions in the myocardial biopsies was also calculated for each

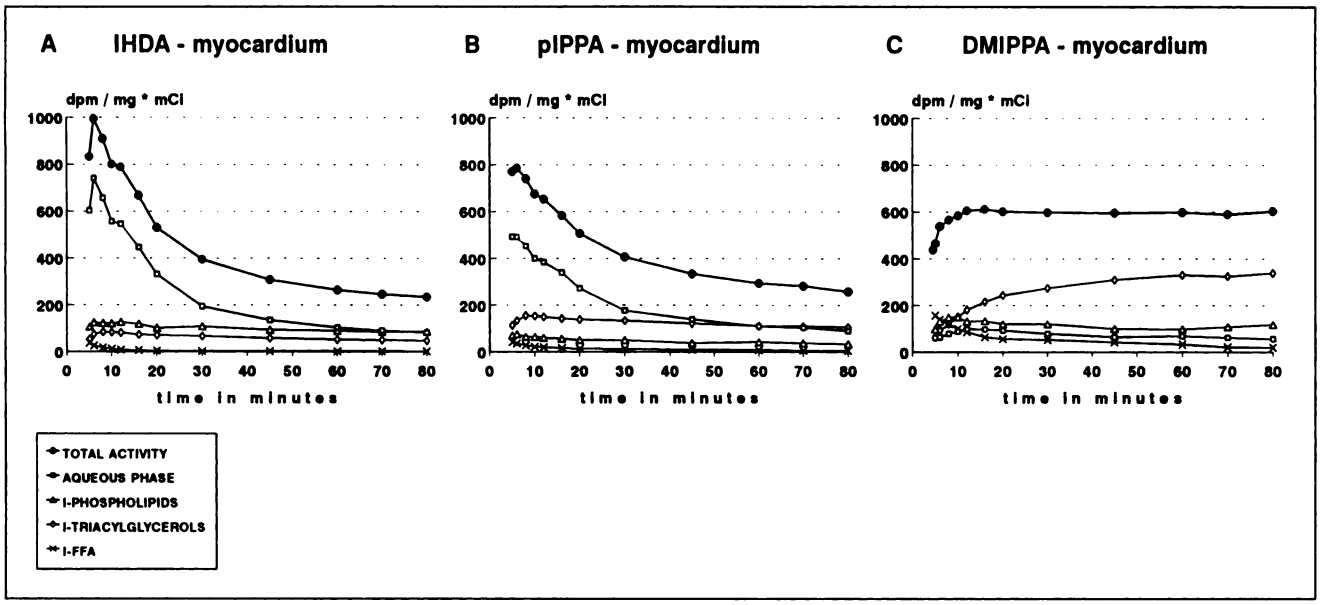


**FIGURE 2.** Time-activity curves of averaged data of (A) IHDA, (B) pIPPA and (C) DMIPPA in myocardial biopsy specimens after simultaneous intravenous injection in six dogs. Total myocardial activity and the fractional activity of the aqueous phase, I-phospholipids, I-triacylglycerols and unmetabolized IFA are expressed as dpm/mg·mCi. The I-diacylglycerols, I-cholesterol esters and pellet fraction are not shown, but are included in the total activity (see text and Table 1).

individual experiment at t = 6, 16, 30 and 80 min and expressed as a percentage of the total myocardial activity. At each sample point, the ratio of total myocardial activity to total blood activity of the three radiolabeled fatty acids was calculated and plotted as a function of time. For calculation of the clearance rates of the total activity and metabolic fractions, averaged data were plotted as a time-activity curve. The time-activity curves of the total activity and the aqueous phase fraction were fitted with a monoexponential plus constant (*13*). The various lipid fractions were fitted with a monoexponential curve. The clearance rates were expressed as half-time values ($t_{1/2}$) in minutes.

### Statistics

Data are presented as the mean and standard deviation values. Student's t-test for paired and unpaired data was used where appropriate. A p value of less than 5% was considered significant.

## RESULTS

### Hemodynamics and Substrate Levels

Before myocardial biopsy specimens were taken, mean arterial blood pressure in the six dogs was 100 ± 21 mmHg and averaged 88 ± 11 mmHg at the end of the experiment. Heart rate was kept at 150 beats per min. The cardiac output was 5.2 ± 0.8 liters/min at t = 0 and 4.1 ± 0.8 liters/min at the end of the experiment.

Prior to injection of the radioiodinated fatty acids, plasma arterial substrate levels for fatty acids, glucose and lactate were 0.54 ± 0.22 mmol/liter, 6.4 ± 1.9 mmol/liter and 1.7 ± 0.5 mmol/liter, respectively. These values were in the normal range and did not change significantly during the assay period.

### IFA Comparison in Myocardium

The time-activity curves of IHDA, pIPPA and DMIPPA in the myocardium are presented in Figure 2. The relative distribution of the metabolites, expressed as a percentage of the total tissue counts at t = 6, 16, 30 and 80 min is listed in Table 1. The results obtained after curve fitting are listed in Table 2.

*Total Myocardial Activity.* The peak myocardial activity of IHDA and pIPPA was reached at the 6th min with values of 995 ± 248 dpm/mg·mCi and 785 ± 197 dpm/mg·mCi, respectively (ns). DMIPPA myocardial activity increased until the 16th min: 612 ± 166 dpm/mg·mCi. The peak DMIPPA activity was significantly lower compared to IHDA total activity at peak ($p < 0.05$). Compared to IHDA and pIPPA, the clearance rate of DMIPPA was prolonged with a half-time value of 287 min versus 11.2 and 13.5 min, respectively.

*Aqueous Phase Fraction.* At t = 6 min, the aqueous phase activity of IHDA was significantly higher than of pIPPA: 74% ± 4% versus 65% ± 5% ($p < 0.05$). The aqueous phase fraction of DMIPPA: 16% ± 9% at t = 16, was significantly lower than of IHDA ($p < 0.0001$) and of pIPPA ($p < 0.001$). The aqueous phase fraction of DMIPPA decreased with a half-time value of 93 min compared to 10.6 and 12.5 min of the IHDA and pIPPA aqueous phase fractions, respectively.

*Phospholipids.* Phospholipids were the principal lipid fraction into which IHDA was incorporated: 13% ± 3% at t = 6 min. The pIPPA phospholipids were significantly lower: 8% ± 2% at t = 6 min ($p < 0.05$ versus IHDA). At t = 6 min, phospholipid activity of DMIPPA (24% ± 5%)

**TABLE 1**
Total Activity and Relative Distribution of Metabolites in Normal Canine Myocardial Tissue

| | Time | | | |
|---|---|---|---|---|
| | 6 min | 16 min | 30 min | 80 min |
| Total activity (dpm/mg/mCi) | | | | |
| IHDA | 995 ± 248† | 667 ± 138 | 395 ± 83† | 235 ± 49† |
| pIPPA | 785 ± 197† | 583 ± 133 | 406 ± 107† | 259 ± 68† |
| DMIPPA | 537 ± 171† | 612 ± 166 | 598 ± 133† | 603 ± 120† |
| Relative distribution (%) | | | | |
| Aqueous phase | | | | |
| IHDA | 74 ± 4*§ | 67 ± 6§ | 50 ± 9§ | 35 ± 7§ |
| pIPPA | 65 ± 5*§ | 60 ± 9‡ | 44 ± 5§ | 37 ± 9§ |
| DMIPPA | 12 ± 8§ | 16 ± 9§ | 11 ± 8§ | 10 ± 8§ |
| l-Phospholipids | | | | |
| IHDA | 13 ± 3*† | 18 ± 2†‡ | 28 ± 6*† | 39 ± 12† |
| pIPPA | 8 ± 2*† | 10 ± 2†‡ | 13 ± 4*† | 14 ± 6† |
| DMIPPA | 24 ± 5*† | 22 ± 4†‡ | 21 ± 5*† | 20 ± 7† |
| l-Triacylglycerols | | | | |
| IHDA | 7 ± 2‡* | 11 ± 6†‡ | 16 ± 10§‡ | 19 ± 13‡† |
| pIPPA | 16 ± 3‡ | 24 ± 7†‡ | 32 ± 9‡ | 38 ± 12†‡ |
| DMIPPA | 18 ± 9* | 34 ± 10† | 45 ± 11§‡ | 55 ± 12†‡ |
| Unmetabolized l-FA | | | | |
| IHDA | 3 ± 2*† | 1 ± 0† | 1 ± 0*† | 1 ± 0* |
| pIPPA | 5 ± 1*† | 3 ± 1† | 3 ± 1† | 2 ± 1* |
| DMIPPA | 27 ± 14†* | 12 ± 7† | 9 ± 7* | 4 ± 2* |
| l-Diacylglycerols | | | | |
| IHDA | <1* | <1‡ | <1* | <1* |
| pIPPA | 2 ± 1* | 3 ± 1† | 4 ± 2* | 3 ± 1* |
| DMIPPA | 1 ± 1 | 1 ± 1† | 2 ± 2* | 1 ± 1* |
| Pellet | | | | |
| IHDA | 2 ± 2* | 2 ± 1* | 4 ± 2* | 5 ± 3* |
| pIPPA | 3 ± 2* | 0 ± 2* | 3 ± 2* | 5 ± 3* |
| DMIPPA | 18 ± 10* | 15 ± 8* | 12 ± 7* | 10 ± 6* |

Total activity in myocardial tissue is presented as dpm/mg·mCi (mean ± s.d.); the relative distribution of metabolites are expressed as a percentage (mean ± s.d.) of the total activity in the biopsy specimen. Radioactivity in the cholesterol ester fractions were negligible and therefore not listed. Paired t-test: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$; §$p < 0.0001$.

was significantly higher than that of IHDA and pIPPA ($p < 0.01$), but was significantly lower than IHDA phospholipids at 80 min ($p < 0.01$). Half-time values of phospholipid clearance rates of IHDA, pIPPA and DMIPPA were 124, 75 and 162 min, respectively.

**TABLE 2**
Clearance Rates of Total Activity and Metabolic Fractions in Normal Canine Myocardium

| | IHDA | pIPPA | DMIPPA |
|---|---|---|---|
| Total activity | 11.2 | 13.2 | 287 |
| Aqueous phase | 10.6 | 12.5 | 93 |
| Phospholipids | 124 | 75 | 162 |
| Triacylglycerols | 86 | 131 | 9.9(inc.) |

Clearance rates are expressed as half-time values in min. Time-activity curves of the total myocardial activity and the aqueous phase fraction are fitted with a monoexponential plus constant according to Eenige van et al. (*12*). The lipid fractions are fitted with a monoexponential curve. Because DMIPPA is gradually incorporated into the triacylglycerols, the half-time value for incorporation (inc.) is given.

*Triacylglycerols.* DMIPPA was gradually incorporated into triacylglycerols with a half-time value of 9.9 min, reaching its maximum at the end of the assay period with a value of 55% ± 12% of the total myocardial activity. DMIPPA triacylglycerols were significantly higher than pIPPA ($p < 0.01$ after $t = 16$ min) and IHDA ($p < 0.05$ at $t = 6$ min). At $t = 6$ min, pIPPA incorporation into triacylglycerols (16% ± 3%) was significantly higher compared to IHDA: 7% ± 2% ($p < 0.001$). Half-time values of triacylglycerol clearance rates of IHDA and pIPPA were 86 and 131 min, respectively.

*Unmetabolized IFA.* A small fraction of unmetabolized IHDA and pIPPA was found, indicating nearly complete metabolic handling of these fatty acids. IHDA levels were significantly lower than those of pIPPA: 3% ± 2% and 5% ± 1%, respectively at $t = 6$ min ($p < 0.05$). In contrast, unmetabolized DMIPPA activity was high: 27% ± 14% at 6 min ($p < 0.01$ versus IHDA and pIPPA), indicating that DMIPPA was metabolized at a relatively low rate.

*Diacylglycerols.* A negligible fraction (1% or less) of IHDA and DMIPPA in diacylglycerols were found. A

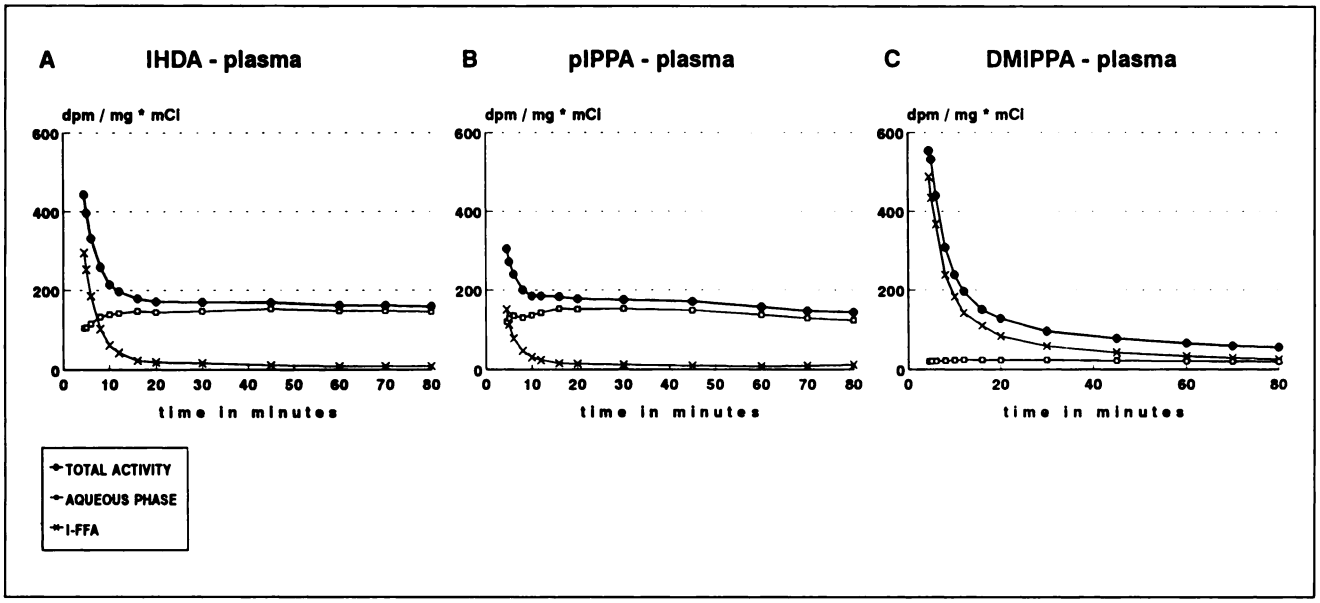


**FIGURE 3.** Time activity curves of averaged data of (A) IHDA, (B) pIPPA and (C) DMIPPA in arterial plasma after intravenous injection in six dogs. Total plasma activity and the distribution over the IFAs and aqueous phase are expressed as disintegrations per milligram plasma per millicurie injected dose (dpm/mg·mCi). The pellet fraction was small and therefore not shown (see text).

small but significant fraction of pIPPA diacylglycerols was found in the biopsy specimens: 2% ± 1% at t = 6 min ($p < 0.05$ versus IHDA) and 4% ± 2% at t = 30 min ($p < 0.05$ versus IHDA and DMIPPA).

*Cholesterol Esters.* None of the three IFAs showed significant incorporation into the cholesterol esters.

*Pellet.* The pellet of IHDA and pIPPA contained less than 5% of total radioactivity. The DMIPPA pellet was significantly higher, containing 10% ± 6% of the total myocardial activity at t = 80 min.

### Comparison of IFAs in Plasma

Figure 3 illustrates the time-course of radioactivity in arterial plasma of IHDA, pIPPA and DMIPPA. Total activity of each IFA rapidly decreased until the 20th min because of dilution and extraction. As demonstrated by TLC, the radioactivity in the organic phase was present entirely in the nonesterified fatty acid fraction. In case of IHDA, a plateau was reached after 20 min which was caused by a constant level of the aqueous fraction (160–170 dpm/mg · mCi). The plasma radioactivity of pIPPA decreased during the assay period because of the decline in radioactivity in the aqueous fraction with a half-time value of 150 min (peak activity: 153 ± 32 dpm/mg·mCi at t = 16 min). DMIPPA plasma activity decreased to a low level compared to the other two iodinated fatty acids, due to formation of relatively small amounts of aqueous phase products (see biopsy specimen data). Maximal aqueous phase activity of DMIPPA in plasma was 24 ± 5 dpm/mg·mCi at t = 20 min. The pellet fraction was always less than 5% of total activity for each IFA.

### Myocardium-to-Blood Ratios

Figure 4 illustrates the changes of myocardial-to-blood activity ratios over time. IHDA and pIPPA ratios were not significantly different. The maximum value for IHDA was 4.1 ± 0.8:1 at t = 12 min and 3.9 ± 1.3:1 at t = 10 min for pIPPA. The values at 80 min were 1.6 ± 0.3:1 and 1.8 ± 0.4:1, respectively. In contrast, the DMIPPA myocardium-to-blood ratios were higher with a maximal value of 12.7 ± 3.7:1 at t = 80 ($p < 0.0001$ versus IHDA and pIPPA).

## DISCUSSION

To be useful as a metabolic tracer, IFAs require the following characteristics:

1. The IFA must be recognized by the myocardium as a natural long-chain fatty acid. Therefore uptake, oxidation or lipid incorporation should be similar to natural fatty acids.
2. The parameters obtained by external scintigraphy (total activity, clearance rate, oxidation size) should provide information on discrete aspects of the metabolism, e.g., uptake, oxidation or lipid incorporation.
3. The scintigraphic data should be useful for diagnostic purposes and provide clear differences between normal and diseased myocardium.

### Uptake, Oxidation and Lipid Incorporation: Comparison with Natural Fatty Acids

Precise measurements of the complex distribution profile of carbon-labeled fatty acids and three IFAs is hardly

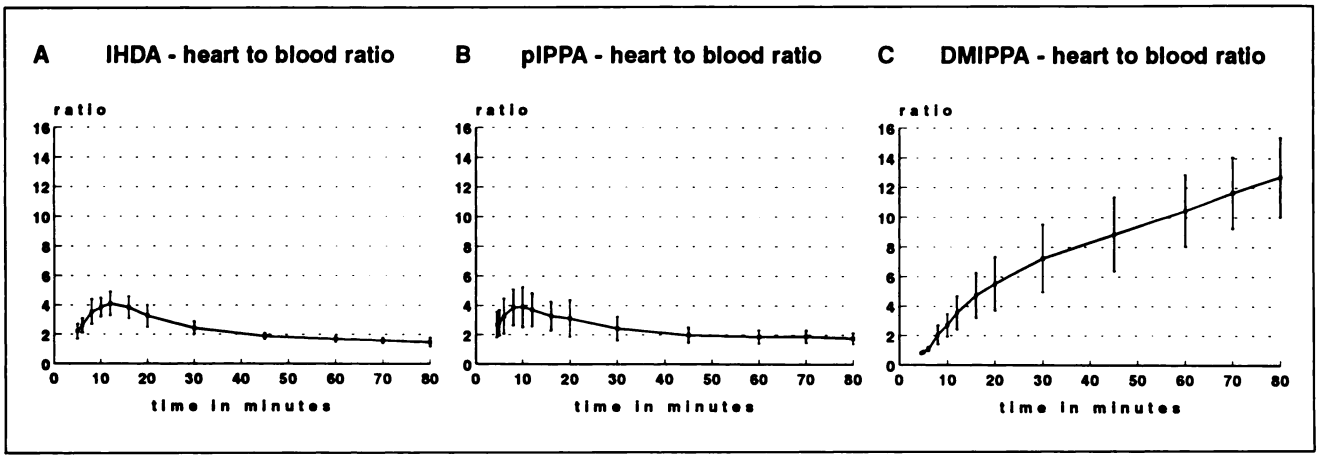


**FIGURE 4.** Myocardium-to-blood ratios (mean ± s.d.) of (A) IHDA, (B) pIPPA and (C) DMIPPA. IHDA and pIPPA show the same pattern and are not significantly different.

possible in the same small biopsy specimens. It could have been done only if total radioactivity or few fractions were measured, or if the biopsy specimens were larger (which is not compatible with a hemodynamically stable animal preparation during the required assay period). There is a limited number of comparative studies between one single radioiodinated fatty acid (either IHDA or pIPPA) and a natural fatty acid, which show good correlation (*14–18*). Therefore, we will discuss the IFA data of this study in relation to data of natural fatty acids reported in the literature.

Uptake of IHDA in rats is similar to stearic acid (*14*), while the uptake of pIPPA is reported to be either higher or lower relative to palmitic acid (*15,16*). In our study, the uptake of IHDA and pIPPA was similar. The initial uptake of DMIPPA was significantly lower than that of IHDA and pIPPA, which is in contrast with results from rats (*9*) where a similar myocardial uptake was found for DMIPPA, BMIPPA and pIPPA.

We observed that oxidation of pIPPA was lower than that of IHDA. Although a direct comparison between the two iodinated fatty acids and natural fatty acids has not been published so far, the data are in line with recent studies in rats, showing that oxidation of IHDA is similar to palmitic acid (*17*) and that oxidation of pIPPA is lower than that of palmitic acid (*16*). In contrast, DeGrado et al. (*18*) found in their compartmental model study in rats that pIPPA oxidation was 1.8 times higher than palmitic acid oxidation. Although DMIPPA was designed to be unoxidizable, small amounts of radioactivity appear in the aqueous phase suggesting that metabolic degradation of DMIPPA cannot be excluded. Preliminary data in ischemic canine myocardium show even lower levels of hydrophilic products compared to controls, suggesting that the formation of this 'metabolite' may be oxidation related. This material does not easily leave the myocardium, since myocardial aqueous phase levels remain 3 to 4 times higher than plasma levels. An $\alpha$-hydroxylated product of DMIPPA, as suggested by Knapp et al. (*9*), might well fit in with these characteristics. Another possible substance in the aqueous phase might be accumulation of the acyl-CoA derivate of DMIPPA because of the hampered oxidative pathway.

Incorporation into the endogenous lipid pools was different. Whereas pIPPA and DMIPPA were primarily incorporated into triacylglycerols, IHDA was mainly incorporated into the phospholipids. Carbon-labeled palmitate studies in dogs (*19,20*) indicated that palmitate was more incorporated into triacylglycerols than phospholipids. However, Van der Vusse et al. (*21*) demonstrated that lipid classes (especially triacylglycerols) in myocardial tissue of normal dogs differed markedly in their relative fatty acid composition. Probably the most accurate comparison that can be made is between the relative composition of natural fatty acids in one lipid class and the radioiodinated analogs of the same chain length: i.e., IHDA versus stearic acid (18:0) and pIPPA versus palmitic acid (16:0). Van der Vusse et al. (*21*) found 16.4% stearic acid and 8.6% palmitic acid in the phospholipids, leading to a calculated ratio of 1.9:1. At peak activity, we found an IHDA-to-pIPPA ratio of 1.7:1 for phospholipids. In triacylglycerols, Van der Vusse et al. (*21*) found 9.6% stearic acid and 21.5% palmitic acid, with a calculated ratio of 0.45:1. We found an IHDA-to-pIPPA ratio of 0.55:1 for triacylglycerols. These findings and results from previous studies on phospholipid distribution (*22,23*) strongly suggest that IHDA behaves like stearic and pIPPA like palmitic acid with respect to incorporation into the myocardial esterified lipid pool.

Compared to IHDA and pIPPA, relatively high levels of unmetabolized DMIPPA were found in the myocardium. Apparently the activation rate of this modified fatty acid is lower compared to the straight-chain fatty acid analogs. Humbert et al. (*24*) questioned the metabolization of methylated fatty acid analogs. The gradual increase in radioactivity recovered in the triacylglycerol

fraction in our study, however, is in good agreement with studies in rats (*25,26*) and suggests that esterification does occur, albeit at a relatively low rate.

In the initial samples, the DMIPPA phospholipid activity was high. A possible explanation that this activity represents 1-acyl-glycerol 3-phosphate, a common precursor of both phospholipids and triacylglycerols which is usually rapidly acylated at the 2-position and thereby converted to phosphatidic acid.

Steric hindrance caused by the two methyl groups in the vicinity of the glycerol backbone may slow down acylation of 1-DMIPPA-glycerol 3-phosphate, leading to accumulation of this metabolite, which would co-migrate with the phospholipid fraction in the used TLC systems. Alternatively, it is possible that phosphatidic acid is formed, which in turn is slowly converted to diacylglycerol by the phosphatidate phosphatase enzyme.

### Relationship Between Biochemistry of Fatty Acids and Scintigraphic Data

Myocardial IHDA radioactivity decreases rapidly with a half-time value of 11.2 min. This rapid decrease is due to the washout of free radioiodine (*27*) with a half-time of 10.5 min (Fig. 2A). These data are consistent with the results of Schön et al. (*28*), who found a half-time value of 9.3 ± 2.8 min. However, these authors suggested that IHDA kinetics could not be compared with natural fatty acids since they found that augmented cardiac work slowed the clearance of $^{123}$I activity. On the other hand, Eenige van et al. (*29*) demonstrated that the calculated oxidation ratio and clearance rates of the scintigram paralleled the biochemical kinetics. Also during hyperlactatemia, hyperglycemia and ischemia, IHDA oxidation has been demonstrated to decrease in favor of esterification (*30–33*). Pacing of the human heart results in a demonstrable increase in uptake and oxidation of IHDA (*34*). Comans et al. found a close correlation between the scintigraphically derived oxidation size of IHDA and oxidation of palmitate (*17*). These data suggest that IHDA uptake and oxidation can be observed by scintigraphy.

Uptake, extraction and clearance rate of pIPPA in different cardiac diseases can also be assessed scintigraphically (*35–40*). The $^{123}$I-labeled pIPPA analog was initially developed to increase the heart-to-background ratio by increasing the myocardial retention due to formation of para-iodobenzoic acid and by renal excretion of iodohippuric acid. Although we did not measure para-iodobenzoic acid directly, it is noteworthy that in our study the clearance rate of myocardial activity ($t_{1/2}$ = 13.5 min) was only slightly slower than that of IHDA ($t_{1/2}$ = 11.2 min). For pIPPA, there is some clearance from the blood ($t_{1/2}$ = 150 min) of the aqueous fraction, most likely containing para-iodobenzoic acid (*6,7*). However, this half-time value is relatively long compared to the acquisition time used in most studies (30–75 min) and therefore this clearance during acquisition is quantitatively insignificant. We also found that the myocardium-to-blood ratios of pIPPA were similar to those of IHDA during the 80-min assay period (Fig. 4A and B). Thus, although lipid pool incorporation is different between IHDA and pIPPA, minor differences exist between scintigraphically obtained variables of IHDA and pIPPA. Because of the relatively rapid myocardial clearance of IHDA and pIPPA, the use of these straight-chain analogs is limited in the assessment of myocardial fatty acid uptake and clearance rates with SPECT. An alternative approach is to inhibit IHDA and pIPPA myocardial clearance rates that occur during exercise or lactate infusion (*32,37,40*). In the near future, new three-head SPECT systems might allow tomographic acquisitions rapid enough (5 min) to provide information on the uptake and clearance of straight-chain fatty acids.

Although uptake of DMIPPA is lower than that of IHDA and pIPPA, regional uptake can be assessed with SPECT because of its prolonged retention in combination with high myocardium-to-blood ratios. These high ratios are consistent with data from rats (*9,45*). Preliminary data of DMIPPA administration to patients confirm the low clearance rate, as we observed a myocardial clearance rate of ±20% after 3 hr.

### IFA Scintigraphy as a Diagnostic Tool

Our study does not contribute to the distinction between normal and diseased myocardium. However, we initiated our studies with the characterization of three IFAs under physiological conditions. A comparative study of these IFAs during hypoxia and ischemia in dogs is in progress. The clinical application of IHDA and pIPPA has been extensively reviewed (*41*). Although no clinical studies of DMIPPA have yet been published, data from studies with 3-monomethyl-branched fatty acids indicate their potential usefulness in evaluating fatty acid uptake with SPECT. Yonekura et al. (*42*) studied the distribution pattern of 1-[$^{14}$C]-3-methylheptadecanoic acid and $^{201}$Tl in hypertensive rat hearts. Uptake of the methyl-branched fatty acid showed a heterogeneous distribution, whereas $^{201}$Tl was homogeneously distributed. The same patterns have been observed with the radioiodinated methyl branched fatty acids, BMIPPA (*43*) and DMIPPA (*44*). Som et al. (*45*) studied cardiomyopathic hamsters and showed that before the onset of severe significant histological changes in the myocardium, glucose utilization and flow were homogeneous whereas the utilization of DMIPPA showed a nonhomogeneous distribution. An improvement of fatty acid utilization was observed when verapamil was administered. Recently Kurata et al. (*46*) demonstrated the same pattern using BMIPPA in patients with hypertrophic cardiomyopathy. Miller et al. (*47*) demonstrated that methylated fatty acid uptake could identify stunned myocardium in ischemic-reperfused myocardium. Fujibayashi et al. (*48*) demonstrated a close correlation between BMIPPA uptake and intracellular ATP levels. Thus, fatty acid uptake and re-

tention could provide useful information about the energy status of the myocardium. Also, preliminary data from our study with regional myocardial ischemia in dogs demonstrated a significant correlation between flow and DMIPPA uptake.

These data suggest that uptake differences in cardiac disease can indeed be visualized with DMIPPA scintigraphy. To assess whether DMIPPA retention is a reliable indicator for natural fatty acid utilization, comparative studies with DMIPPA and palmitate under different metabolic and pathophysiologic conditions are needed.

In conclusion, differences in the uptake, oxidation and lipid distribution of IHDA, pIPPA and DMIPPA are found in normal canine myocardium. Uptake and oxidation are variables that can be assessed by scintigraphy and are similar for IHDA and pIPPA. Lipid distributions of IHDA and pIPPA are different yet resemble the $^{18}C$ and $^{16}C$ analogs, respectively. The myocardial activity of DMIPPA does not show significant redistribution and myocardium-to-blood ratios reach values of greater than 10:1. These properties suggest that DMIPPA is a promising tracer for quantification of myocardial fatty acid uptake by SPECT.

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