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Long Chain Fatty Acid Uptake In Vivo: Comparison of [¹²⁵I]-BMIPP and [³H]-Bromopalmitate

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Abstract

Insulin resistance is characterized by increased metabolic uptake of fatty acids. Accordingly, techniques to examine in vivo shifts in fatty acid metabolism are of value in both clinical and experimental settings. Partially metabolizable long chain fatty acid (LCFA) tracers have been recently developed and employed for this purpose: [9,10-³H]-(*R*)-2-bromopalmitate ([³H]-BROMO) and [¹²⁵I]-15-(*p*-iodophenyl)-3-*R,S*-methylpentadecanoic acid ([¹²⁵I]-BMIPP). These analogues are taken up like native fatty acids, but once inside the cell do not directly enter β -oxidation. Rather, they become trapped in the slower processes of ω and α -oxidation. Study aims were to (1) simultaneously assess and compare [³H]-BROMO and [¹²⁵I]-BMIPP and (2) determine if tracer breakdown is affected by elevated metabolic demands. Catheters were implanted in a carotid artery and jugular vein of Sprague–Dawley rats. Following 5 days recovery, fasted animals (5 h) underwent a rest ($n = 8$) or exercise ($n = 8$) (0.6 mi/h) protocol. An instantaneous bolus containing both [³H]-BROMO and [¹²⁵I]-BMIPP was administered to determine LCFA uptake. No significant difference between [¹²⁵I]-BMIPP and [³H]-BROMO uptake was found in cardiac or skeletal muscle during rest or exercise. In liver, rates of uptake were more than doubled with [³H]-BROMO compared to [¹²⁵I]-BMIPP. Analysis of tracer conversion by TLC demonstrated no difference at rest. Exercise resulted in greater metabolism and

excretion of tracers with ~37% and ~53% of [125 I]-BMIPP and [3 H]-BROMO present in conversion products at 40 min. In conclusion, [3 H]-BROMO and [125 I]-BMIPP are indistinguishable for the determination of tissue kinetics at rest in skeletal and cardiac muscle. Exercise preferentially exacerbates the breakdown of [3 H]-BROMO, making [125 I]-BMIPP the analogue of choice for prolonged (>30 min) experimental protocols with elevated metabolic demands.

Keywords

Tracer; Kinetics; Thin layer chromatography; Uptake; Clearance

Introduction

Abnormalities in lipid trafficking and uptake are a hallmark of numerous metabolic disease states including obesity, type 2 diabetes and atherosclerosis. To assess lipid kinetics in these states, partially metabolizable long chain fatty acid (LCFA) tracers have been developed for use in vivo [1–7]. Two such tracers are [9,10- 3 H]-(*R*)-2-bromopalmitate ([3 H]-BROMO) and [125 I]-15-(*p*-iodophenyl)-3-*R*,*S*-methylpentadecanoic acid ([125 I]-BMIPP). Both analogues are taken up by tissues like native substrates however, once inside the cell, they become trapped in various stages of ω or α -oxidation. As a result, the analogues remain in the tissue allowing their quantification by specific activity.

Developed and extensively tested by Oakes et al. [8], [3 H]-BROMO shows excellent retention in the majority of tissues examined. Studies employing this tracer were the first to effectively show increased efficiency of tissue LCFA uptake in a model of dietary induced insulin resistance [9]. BMIPP was developed by Knapp et al. [10–12] and has been primarily used for cardiac imaging with an [123 I] label. Using single photon emission computed tomography (SPECT) defects in fatty acid uptake by the heart are imaged and are indicative of ischemia or tissue injury [5, 13–15]. Like [3 H]-BROMO, the tracer has also proven to be a powerful tool in evaluating various pharmacological treatments on cardiac metabolism [16–19].

To date, numerous studies have individually assessed the metabolism of these fatty acid tracers in vivo and some comparisons between different tracers have been reported [1]. However, studies directly comparing [125 I]-BMIPP and [3 H]-BROMO in specific tissues under varied metabolic conditions have not been performed. Therefore, the aim of the present study was to assess and compare [125 I]-BMIPP and [3 H]-BROMO in vivo. The effects of enhanced LCFA metabolism due to exercise on tissue retention of these tracers was examined. Together, these studies will provide needed information on the use of [125 I]-BMIPP and [3 H]-BROMO, and their applicability to the study of metabolism.

Experimental Methods

Animals

Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN) were housed individually and maintained at 23°C on a 0600–1800 hours light cycle. Rats were fed standard chow ad

libitum (Purina, Nestlé, St Louis, MI) and given free access to water. The rats were housed under these conditions for ~1 week, by which time their weights had reached ~330 g. Following weight gain, rats were randomly divided into rest and exercise groups ($n = 8$ per group). All procedures were approved by the Vanderbilt University Animal Care and Use Subcommittee and followed National Institutes of Health guidelines for the care and use of laboratory animals.

Surgical Procedures

Surgical procedures were performed as previously described for arterial and venous catheterizations [20]. Briefly, animals were anaesthetized with a 50:5:1 vol/vol/vol mixture of ketamine, rompun, and acepromazine, and the left common carotid artery and right jugular vein were catheterized with PE50 catheters, which were exteriorized and secured at the back of the neck, filled with heparinized saline (150 U/ml), and sealed with a stainless steel plug. Immediately post-surgery, each animal received 75 mg/kg of ampicillin subcutaneously to prevent infection. After surgery, animal weights and food intake were monitored daily.

Isotopic Analogues

(*p*-iodophenyl)-3-*R,S*-methylpentadecanoic acid was a kind gift from Oak Ridge International Laboratories (Oak Ridge, TN). Radioiodination was performed according to the manufacturer's suggested protocol. Briefly, (*p*-iodophenyl)-3-*R,S*-methylpentadecanoic acid was heated in the presence of Na[¹²⁵I] solution (740 MBq/200 µl), propionic acid, and copper (II) sulfate. Na₂S₂O₃ was then added and the organic phase ether extracted and sequentially back extracted with saturated NaHCO₃ and water. After evaporation, the [¹²⁵I]-BMIPP was solubilized using sonication into ursodeoxycholic acid. The initial concentration of [¹²⁵I]-BMIPP was 1.05 µCi/µl. However, due to the short half life of [¹²⁵I]-BMIPP, the administered dose was volumetric. A dose of 100 µl was administered and the activity of the tracer was determined for each individual animal and corrected for any decay. To limit tracer decay, all studies were performed within 30 days of synthesis.

[³H]-BROMO was prepared by American Radioactive Chemicals Co (St Louis, MO) from palmitic acid as previously described [8]. The final concentration was 1 µCi/µl. On the day of the experiment, 20 µl of [³H]-BROMO was evaporated and reconstituted in 100 µl of saline containing 0.35 mg/ml rat albumin (Sigma Chemical, St Louis, MO). This solution was added to 100 µl of [¹²⁵I]-BMIPP in ursodeoxycholic acid. In total, [³H]-BROMO, 19.5 µCi was administered to each animal. On the day of the experiment, 5 µl of infusate was retained for normalization of radioactivity while the remaining 195 µl was administered to the animal.

Resting Studies

On the morning of the study, rats were fasted for 5 h in a clear Tupperware (2 l) container lined with bedding. Approximately 1 h prior to the experiment, catheters were flushed with heparinized saline (10 U/ml) and connected to PE50 and Silastic tubing for infusions and sampling. Rats were then placed in the Tupperware container until the commencement of the experimental protocol. Throughout the experimental protocol rats were conscious and

unrestrained. In total the experimental protocol was 40 min in duration. Prior to tracer infusions, a basal blood sample were obtained (100 µl) for the measurement of isotopic analogues, plasma glucose, insulin and nonesterified fatty acids (NEFA). To prevent declines in hematocrit, the erythrocytes taken prior to isotopic analogue infusion were washed in saline and re-infused shortly after each sample was taken. At 0 min, an instantaneous bolus of [125 I]-BMIPP and [3 H]-BROMO was administered and additional plasma samples (200 µl) were obtained at 2, 5, 10, 15, 25, and 40 min for the measurement of [125 I]-BMIPP, [3 H]-BROMO, NEFA and plasma glucose. Following the final blood sample, rats were anesthetized with pentobarbital sodium and skeletal muscle (gastrocnemius), liver, heart, brain and epididymal fat were rapidly excised, rinsed in saline to remove excess blood, freeze clamped in liquid nitrogen and frozen at -80°C until further analysis.

Exercise Studies

Two days prior to the protocol, rats in this treatment were acclimated by running on a motorized treadmill for 10 min at 0.6 mi/h. On the morning of the study, rats were fasted for 5 h in a clear Tupperware (2 l) container lined with bedding. Approximately 1 h prior to the experiment, catheters were flushed with heparinized saline (10 U/ml) and connected to PE50 and silastic tubing for infusions and sampling. Rats were then placed in the treadmill until the commencement of the experimental protocol. Following basal blood sampling, rats were run on the treadmill at 0.6 mi/h and samples were obtained as in the resting protocol. This exercise intensity is moderate for rats. At 40 min, rats were anesthetized and tissue samples were obtained as previously described.

Plasma Analysis

Plasma NEFAs were measured spectrophotometrically by an enzymatic colorimetric assay (Wako NEFA C kit, Wako Chemicals Inc., Richmond, VA). Plasma glucose concentrations were measured by the glucose oxidase method using an automated glucose analyzer (Beckman Instruments, Fullerton, CA). [125 I]-BMIPP and [3 H]-BROMO were measured in the same plasma sample (15 µl). Plasma was counted for [125 I]-BMIPP using a Beckman Gamma 5500 counter (Beckman Instruments, Fullerton, CA). Following this, 100 µl of 50% ethanol was added to the sample and ^3H radioactivity was counted after addition of fluor (10 ml; Ultimate Gold, Packard Bioscience, Boston, MA.) using a Packard Tri-Carb 2900TR Liquid Scintillation Analyzer (PerkinElmer, Boston, MA). In addition, plasma was also analyzed by thin layer chromatography (TLC) by methods that were derived from Kropp et al. [21]. Here, individual plasma time points were examined by TLC from a representative animal in each treatment group. TLC plates reflecting plasma samples were segmented into 1 cm sections and analyzed for tracer and tracer conversion products. Each plate was examined for radioactivity. Significant radioactivity appearing in minor lipid fractions over time was considered evidence of tracer breakdown, although the exact chemical nature of each product was not identified. Tracer conversion or breakdown was calculated as a ratio of total activity appearing on the plate for each time point.

Tissue Analysis

Total [125 I] radioactivity in tissues was determined on sections of whole tissue (~100 mg) using a Beckman Gamma 5500 counter (Beckman Instruments, Fullerton, CA). Following

this, total lipid was extracted from the tissue using a modified Folch–Lees extraction [22]. The [^{125}I] radioactivity in this fraction was then reassessed before 10 ml of flour was added to samples and then ^3H radioactivity determined by liquid scintillation counting (Packard TRI-CARB 2900TR, Packard, Meriden, CT) with Ultima Gold (Packard) as scintillant. The relationship between gamma radioactivity and beta emissions using this specific process and counter has been previously established in our laboratory. This relationship was used to correct ^3H radioactivity for beta-emissions originating from [^{125}I] radioactivity in both tissue and plasma samples [3]. In addition, lipid was extracted from a portion of each tissue (~100 mg) from four rats in both the resting and exercise protocols using a Folch–Lees extraction [22]. Lipid fractions were then separated by TLC [23]. Plate segments were subsequently separated and individually counted. Tissues plates were separated based on lipid fraction (phospholipid, mono-diglyceride, free fatty acids and triglyceride).

Calculations

Plasma Kinetics—Identical equations were used for the determination of [^{125}I]-BMIPP and [^3H]-BROMO kinetics. Plasma tracer (p) kinetics are based on the disappearance of tracer from the plasma over time. Movement of the tracer out of the plasma pool into tissues is denoted by clearance (K_p , Eq. 1). When K_p is expressed in terms of tracee or mean LCFA concentration as measured by an enzymatic assay, the measure is termed uptake (R_p , Eq. 2). Finally, if K_p is expressed as a fraction of the original tracer dose administered (D), the resultant expression is metabolic clearance rate or MCR_p . Equations 1–3 are calculated independently for [^{125}I]-BMIPP and [^3H]-BROMO. Equations employed were defined as follows where $\int_0^t$ represents integral over the time between 2 and 40 min.

$$K_p = \int_0^t \left[[^{125}\text{I}] - \text{BMIPP or } [^3\text{H}] - \text{BROMO} \right]_p dt \quad (1)$$

$$R_p = K_p [\text{LCFA}]_p \quad (2)$$

$$\text{MCR}_p = \frac{D}{\int_0^t \left[[^{125}\text{I}] - \text{BMIPP or } [^3\text{H}] - \text{BROMO} \right]_p dt} \quad (3)$$

Tissue Kinetics—Rates of tissue LCFA clearance (K_t , Eq. 4) and metabolic indices (R_t , Eq. 5) were calculated from the accumulation of [^{125}I]-BMIPP and [^3H]-BROMO in tissues (t) relative to the integral of the plasma (p) concentration following the instantaneous bolus. These measurements follow from Eqs. 1–3 and have been previously described [3, 9].

$$K_t = \frac{\left[[^{125}\text{I}] - \text{BMIPP or } [^3\text{H}] - \text{BROMO} \right]_t}{\int_0^t \left[[^{125}\text{I}] - \text{BMIPP or } [^3\text{H}] - \text{BROMO} \right]_p dt} \quad (4)$$

$$R_t = K_t[\text{LCFA}]p \quad (5)$$

A one-way repeated measures analysis of variance (ANOVA) was performed to compare differences between [^{125}I]-BMIPP and [^3H]-BROMO within specific tissues. To determine differences over time for blood glucose and NEFA, a two-way repeated measures ANOVA was performed. To establish differences within ANOVA, a Tukey post hoc test was used. Significance levels of $p < 0.05$ were employed, and data are reported as means $\pm$ standard error of the mean (SEM).

Results

Animal Characteristics

Baseline animal characteristics for both rest and exercise experiments are outlined in Table 1. Blood glucose remained stable in the rest group (7.7 ± 0.3 mM at 40 min) while it steadily increased in the exercise group to 11.5 ± 0.6 mM at the end of the protocol ($p < 0.05$). Plasma NEFA levels remained stable with average values of 0.63 ± 0.06 mM and 0.56 ± 0.05 mM for rest and exercise studies at the end of the experimental protocol ($p > 0.05$). All animals in the exercise protocol were able to successfully complete the required 40 min of exercise.

Metabolic Clearance and Uptake

Whole body metabolic clearance in the resting state was set to an arbitrary value of 1.0 for each tracer for comparison to the exercise treatment. Results show comparable increments in fatty acid clearance for each tracer with exercise (Fig. 1). Tissues (skeletal muscle, heart, liver, adipose tissue) were examined for rates of fatty acid uptake (R_t , $\mu\text{mol}/100$ g/min). Absolute values are shown in Fig. 2. No quantitative difference between [^{125}I]-BMIPP and [^3H]-BROMO R_t values were noted during the resting protocol with the exception of the liver. In the liver, rates of R_t calculated using [^3H]-BROMO was more than double that calculated using [^{125}I]-BMIPP.

Tracer Correlations

Correlations of tissue fatty acid uptake between [^{125}I]-BMIPP and [^3H]-BROMO are plotted in Fig. 3. R^2 values for resting and exercise samples are plotted individually, and an aggregate value also presented. Results of individual muscle types for rest and exercise studies were at least moderately correlated, with aggregate R^2 values of 0.39, 0.55, 0.26, and 0.56 for the soleus, vastus lateralis, gastrocnemius and heart respectively. In contrast, data from liver and adipose tissue are poorly correlated between tracers with both treatments.

Plasma Analysis

Analysis of tracer distribution in resting animals by TLC demonstrated no observable differences between [^{125}I]-BMIPP and [^3H]-BROMO at rest. Each tracer showed the expected exponential decay pattern and similar rates of fractional conversion in this state (Table 2). Analysis of fractional conversion during exercise was comparatively greater than

exercise at all time points analyzed. Furthermore, results show conversion of [^3H]-BROMO to exceed that of [^{125}I]-BMIPP in the latter stages (>20 min) of the experiment (Table 2).

Tracer Tissue Distribution

The intracellular fate of tracers were analyzed by TLC. Results demonstrated tracers to reside in three distinct fractions (Table 3). The first fraction contained phospholipid, monoglyceride, and diglyceride (PL + MG + DG) while others analyzed free fatty acids (FFA), triglyceride (TG). Analysis showed the majority of radioactivity resided in the PL + MG + DG fraction followed by FFA and finally TG. Differences between tracer distributions were minimal. At rest, a lower fraction of [^{125}I]-BMIPP was found in TG in cardiac muscle. During exercise, lower [^{125}I]-BMIPP was found in cardiac FFA and skeletal muscle TG.

Discussion

The purpose of this study was to assess and compare [^3H]-BROMO and [^{125}I]-BMIPP during rest and a state of accelerated fatty acid metabolism (moderate exercise). Tracers were simultaneously administered so that direct comparisons of tissue fatty acid uptake could be made. Results show good agreement between [^3H]-BROMO and [^{125}I]-BMIPP for cardiac and skeletal muscle during rest and exercise. In contrast, liver and epididymal fat pads showed poor correlations under both conditions. At rest, rates of liver [^3H]-BROMO uptake were more than double those of [^{125}I]-BMIPP.

Conceptually, both agents are regarded as “generic” long chain fatty acid tracers. The close agreement between these tracers in muscle tissue is reassuring in terms of this usual simplification. There may be tissue specific differences related to precise molecular structure of both fatty acids and fatty acid tracers that are reflected in the differences in adipose and liver. An increase in hepatic extraction and breakdown of [^3H]-BROMO compared to [^{125}I]-BMIPP may also play a role. Evidence of increased hepatic [^3H]-BROMO breakdown has been previously reported [8]. Intravenous administration of [^3H]-BROMO to rodents found liver to have a retention rate of only 77% compared to skeletal muscle that retained over 90% of tracer during 16 min of infusion [8]. Given these and the present findings, it is reasonable to hypothesize that [^{125}I]-BMIPP retention rates may most closely reflect actual liver LCFA uptake. For epididymal fat, we show absolute rates of fatty acid uptake to be similar between tracers. However, correlation of individual animals between [^3H]-BROMO and [^{125}I]-BMIPP for this tissue yielded poor results. This discrepancy is likely due to the low rate of fatty acid uptake and tracer detection in this tissue.

To examine fractional conversion of each tracer by tissue, detailed analysis of tracer distribution in plasma over time was conducted by TLC. A known conversion product for [^3H]-BROMO is $^3\text{H}_2\text{O}$. Previous analysis of the tracer show conversion to be ~5% of the dose at 16 min in sedentary rats [8]. This value compares favorably with the present study that estimates a conversion of ~4% at 15 min at rest. Like [^3H]-BROMO, [^{125}I]-BMIPP becomes trapped in α -oxidation [24]. End products of this reaction yield CO_2 , a fatty acid shortened by one carbon and a methyl substitution at the second carbon, which in the case of [^{123}I]-BMIPP and [^{131}I]-BMIPP is 2-(ρ -iodophenyl) acetic acid (IPC₂) [21]. Previous analysis of fractional conversion of [^{123}I]-BMIPP show ~11% of injected dose is released at

60 min in humans while perfusion of isolated rat hearts have a conversion of ~12% following 3 h. In the present study, we show conversion of BMIPP to be ~12% of total radioactivity at 40 min. Comparison of [^{125}I]-BMIPP and [^3H]-BROMO during rest, show comparable levels of conversion over time. Given this, either tracer is suitable for resting studies.

Elevation of metabolic demands by moderate exercise resulted in increases fatty acid uptake in skeletal and cardiac muscle for both [^{125}I]-BMIPP and [^3H]-BROMO. Generally, tissue extraction of fatty acids doubled with exercise. This finding confirms previous observations of Oakes and colleagues [8] who have shown [^3H]-BROMO is not affected by metabolic (oxidative vs. nonoxidative) status in skeletal muscle. Of note, differences in liver fatty acid uptake at rest were not observed during exercise. This may be due to a diversion of blood flow away from this organ during exercise. In addition to increasing tracer tissue uptake, exercise also resulted in greater tissue excretion of tracers at all time points measured. At 40 min, 37 and 53% of the initial tracer dose were present in conversion products for [^{125}I]-BMIPP and [^3H]-BROMO respectively. This excretion is due to elevated α and ω -oxidation of fatty acids with exercise or simply back diffusion. However, despite increasing fractional conversion with exercise, we show good agreement between tracers in cardiac and skeletal muscle.

From a technical perspective, [^3H]-BROMO has advantages primarily related to the label-induced limitations of [^{125}I]-BMIPP. These include its shorter half-life, elevated biological risk and synthesis. [^{125}I]-BMIPP has a half life of 60 days compared to [^3H]-BROMO which is 12 years. Given this, corrections for tracer decay with [^{125}I]-BMIPP need to be considered, when studies extend over days to weeks. In the present study, a single batch of [^{125}I]-BMIPP was employed and studies occurred over a 1-month period. All results were scaled to include corrections for tracer decay. Another consideration involves the biological risk involved with working these isotopes. [^{125}I]-BMIPP is a gamma and X-ray emitter and although used in low doses, still poses a greater hazard than [^3H]-BROMO which is a lower energy beta emitter. Finally, the labelling for [^3H]-BROMO is complex but the compound is currently commercially available, while [^{125}I]-BMIPP is straightforward to label but requires specific precautions related to iodination, and currently must be performed in an institutional setting. Finally, in studies where multiple tracers are employed, there may be specific limitations related to other radioactive labeled compounds in concurrent use. These issues must be considered prior to commencing studies with either tracer.

In conclusion, this study directly compares isotopic analogs for the measurement of fatty acid kinetics in vivo. Results show both analogues are effective for the measurement of FFA uptake and clearance in plasma. We showed a high correlation between tracers for cardiac and skeletal muscle. However, in both the liver and adipose tissue, derived rates of uptake diverged depending on the specific tracer. As a result, studies employing these or other fatty acid tracers in these tissues must be interpreted with caution. Generally, technical considerations argue for [^3H]-BROMO. However, when studies are prolonged (>20 min) and employ experimental manipulations requiring elevated metabolism, we show the preferable analog to be [^{125}I]-BMIPP due to its lower rates of tissue excretion.

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Abbreviations

[¹²⁵ I]-BMIPP	[¹²⁵ I]-15-(<i>p</i> -Iodophenyl)-3- <i>R,S</i> -methylpentadecanoic acid
([³ H]-BROMO)	[9,10- ³ H]-(<i>R</i>)-2-Bromopalmitate
<i>K_t</i>	Tissue long chain fatty acid clearance
LCFA	Long chain fatty acid
NEFA	Nonesterified fatty acid
<i>R_t</i>	Index of tissue long chain fatty acid uptake
TLC	Thin layer chromatography

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Fig. 1. Relative change in whole body LCFA clearance rates (MCR) for $[^3\text{H}]\text{-BROMO}$ (*top panel*) and $[^{125}\text{I}]\text{-BMIPP}$ (*bottom panel*) from rest to exercise. Calculations are based on the measurement of radioactivity in the plasma over time. As tracer moves from the plasma into tissues, the rate of decay for each tracer can be quantified. Resting values for each tracer were set to an arbitrary value of one. Values represent means $\pm$ SEM

Fig. 2. Tissue fatty acid uptake ($\mu\text{mol}/100\text{ g}/\text{min}$) for [125I]-BMIPP (filled bars) and [3H]-BROMO (empty bars). Tissues were collected following 40 min of rest or moderate intensity exercise. * Indicates a significant difference between [3H]-BROMO and [125I]-BMIPP within an experimental protocol. † Indicates a significant difference between rest and exercise for a given tracer. Values represent means $\pm$ SEM

Fig. 3.

Comparison of fatty acid uptake rates between [^3H]-BROMO (y axis) and [^{125}I]-BMIPP (x axis) for individual tissue measurements. Values for resting (*open circles*) and exercise (*closed circles*) are shown along with their corresponding R^2 values for rest, exercise and total aggregate are shown

Table 1

Baseline characteristics of animals in the sedentary and exercise experiments

	<i>n</i>	Weight (g)	Recovery (days)	Glucose (mM)	NEFA (mM)	Hct 0 (%)	Hct 40 (%)
Rest	8	330 ± 10	7.8 ± 0.9	7.3 ± 0.2	0.76 ± 0.11	47 ± 1	43 ± 1
Exercise	8	340 ± 12	8.3 ± 0.4	7.5 ± 0.2	0.73 ± 0.11	46 ± 1	41 ± 1

Recovery (days) reflects the number of days between surgery and the experiment. Basal plasma glucose (glucose) and non-esterified fatty acids (NEFA) are shown along with starting and ending hematocrit (Hct) levels

Table 2

Estimated fractional tracer conversion of [^3H]-BROMO and [^{125}I]-BMIPP in plasma over time during rest and exercise

	2 (%)	5 (%)	10 (%)	15 (%)	25 (%)	40 (%)
Rest						
[^{125}I]-BMIPP	1.2	6.3	12.4	14.3	11.8	11.4
[^3H]-BROMO	1.0	4.0	9.2	12.7	10.5	10.4
Exercise						
[^{125}I]-BMIPP	4.6	10.4	14.6	17.3	24.7	37.0
[^3H]-BROMO	5.3	10.6	21.2	40.4	53.5	52.9

Results represent the % of radioactivity in breakdown products versus [^3H]-BROMO and [^{125}I]-BMIPP as assessed by thin layer chromatography

Table 3

Incorporation of [^{125}I]-BMIPP and [^3H]-BROMO into various lipid fractions in tissues at rest ($n = 5$) and exercise ($n = 5$) as determined by thin layer chromatography

Tissue	Lipid fraction	[^3H]-BROMO	[^{125}I]-BMIPP
Rest			
Heart	PL + MG + DG	$45.5 \pm 7.3^{\#}$	$49.3 \pm 9.6^{\#}$
	FFA	$29.9 \pm 8.7^{\#}$	41.4 ± 10.1
	TG	32.7 ± 4.1	$9.3 \pm 2.3^*$
Skeletal muscle	PL + MG + DG	50.7 ± 10.9	45.0 ± 11.7
	FFA	36.7 ± 7.1	41.2 ± 13.0
	TG	15.6 ± 6.1	14.0 ± 4.3
Liver	PL + MG + DG	59.6 ± 6.3	37.9 ± 11.5
	FFA	22.2 ± 6.3	49.2 ± 13.3
	TG	19.2 ± 2.5	12.9 ± 2.6
Exercise			
Heart	PL + MG + DG	69.7 ± 8.9	83.4 ± 9.5
	FFA	13.0 ± 1.7	$2.4 \pm 1.0^*$
	TG	17.3 ± 7.6	11.2 ± 6.6
Skeletal muscle	PL + MG + DG	43.7 ± 6.1	67.4 ± 7.8
	FFA	34.9 ± 5.5	22.6 ± 5.7
	TG	24.4 ± 5.0	$6.3 \pm 1.5^*$
Liver	PL + MG + DG	54.0 ± 8.6	49.4 ± 15.4
	FFA	25.0 ± 8.7	39.4 ± 14.7
	TG	21.0 ± 8.8	11.2 ± 5.3

Values are expressed as a percentage of total radioactivity and represent means $\pm$ SEM.

PL phospholipid, *MG* monoglyceride, *DG* diglyceride, *FFA* free fatty acids, *TG* triglyceride.

* Represents a significant difference between [^{125}I]-BMIPP and [^3H]-BROMO within an experimental protocol

[#] Represents a significant difference with [^{125}I]-BMIPP or [^3H]-BROMO between rest and exercise protocols