# Myocardial Metabolism of Free Fatty Acids

## Studies with $^{14}C$-labeled Substrates in Humans

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## Abstract

**Free fatty acids are considered to be the major energy source for the myocardium. To investigate the metabolic fate of this substrate in humans, 24 subjects underwent coronary sinus and arterial catheterization. 13 subjects were healthy volunteers and 11 subjects had symptoms of ischemic heart disease. [1-$^{14}C$]oleate or [1-$^{14}C$]palmitate bound to albumin was infused at a constant rate of 25 $\mu$Ci/h. Oxidation was determined by measuring the $^{14}CO_2$ production. The data demonstrated that a high percentage (84±17%) of the palmitate and oleate extracted by the myocardium underwent rapid oxidation. A highly significant correlation was present between the arterial level and the amount oxidized ($r = 0.82$, $P < 0.001$ for palmitate; $r = 0.77$, $P < 0.001$ for oleate). The isotope extraction ratio was greater than the chemical extraction ratio. This difference of 6±2 nmol/ml of blood in the young normal subjects was significantly less than the 12±4 nmol/ml observed in the ischemic heart disease patients ($P < 0.001$).**

## Introduction

Lipids are well recognized as a source of energy for the myocardium. In 1941 Cruickshank and Kosterlitz (1) reported that the heart used endogenous stored fats and exogenous fatty acids when glucose was not available in the perfusion media. Several years later, Lehninger (2) found that the rat heart contained the enzymes essential for complete beta oxidation of fatty acids. Shipp and his colleagues (3) in 1961 demonstrated for the first time that palmitate was preferentially oxidized by the myocardium when both glucose and palmitate were present in a perfused isolated rat heart preparation.

Since that time, many studies have assessed myocardial metabolism in vivo in animals and humans by measuring the chemical concentration of free fatty acids in the artery and coronary sinus (4–15). The arterial-coronary sinus chemical differences suggested that free fatty acids (fatty acids bound to albumin) were the preferred myocardial substrate in the fasting, resting state. The metabolic fate of free fatty acids extracted by the myocardium was not determined but the low respiratory quotient of the myocardium suggested that fat was the major substrate for oxidative metabolism (4, 7, 11, 13–15). If all the fatty acids extracted by the myocardium underwent immediate oxidation, free fatty acids would account for 66–100% of the oxidative metabolism. Recently positron emission tomography with [$^{11}C$]palmitic acid has been used to investigate free fatty acid metabolism in normal and ischemic myocardium (16, 17). The time-activity curves of the $^{11}C$ tracer are often equated with oxidation of the fatty acid; however, the precise metabolic fate of free fatty acids in the myocardium has not been defined in humans.

Accordingly, we studied young healthy male volunteers and male patients with documented coronary artery disease to define the metabolic fate of free fatty acids in the human myocardium. [1-$^{14}C$]palmitate or [1-$^{14}C$]oleate bound to albumin was used as a tracer. The myocardial oxidation of the individual free fatty acids was assessed by measuring the myocardial production of $^{14}CO_2$. Thin layer chromatography/gas chromatography was used to measure the specific activity of the individual free fatty acids.

## Methods

*Subject selection.* 13 young healthy male volunteers between the ages of 18 and 27 (mean age 22±3 yr) were selected for group I. These volunteers underwent a complete history, physical examination, and laboratory tests, including an electrocardiogram, complete blood count, liver function tests, blood urea nitrogen, creatinine, fasting glucose, and urinalysis. Each subject had to complete at least stage V of the standard Bruce protocol (18) and reach 95% of his maximal predicted heart rate (19) on the treadmill exercise test. In addition, the subject could not be < 5% or > 15% of his predicted weight. Subjects with a history of chronic drug use or smoking were excluded. If there was no evidence of cardiac or any major systemic illness and all the laboratory tests were within normal limits, the volunteer was accepted as a subject. The examination and exercise test were performed at least 1 wk before the metabolic study.

Group II consisted of 11 male patients who underwent clinically indicated selective coronary angiography for chest pain typical of angina. The age range was 33–70 yr (mean age 52±13 yr). Two of the 11 patients were taking only nitroglycerine for episodes of chest pain. The remaining nine patients were receiving beta-adrenergic blockade therapy, and eight of these were also receiving long-acting nitrates. There was no alteration in drug therapy or dosage before this study. No patient included in this study had unstable or rest angina. All 11 patients underwent left ventriculography and selective coronary angiography; however, the metabolic procedure always preceded angiography (20).

The protocol was approved by the Committee on Human Research of the University of California and the Veterans Administration Medical Center at San Francisco, CA. The use of radioisotopes was approved by the Radiation Safety Committee of the Veterans Administration Medical Center. Each subject was informed of the nature, purpose, and possible risks involved in the study before written consent was obtained.

*Protocol.* All subjects fasted for 10–15 h before the procedure. A local anesthetic (2% lidocaine) was used to perform a venous cutdown; a 7F Wilson Webster thermodilution-flow coronary sinus catheter was inserted into an anteromedial antecubital vein and positioned under fluoroscopy in the mid-to-anterior region of the coronary sinus to avoid reflux (21). In addition, no patient included in the study had evidence of right heart failure or a mean right atrial pressure > 5 mmHg. To verify that the catheter was in the coronary sinus, pressure and $O_2$ saturation were mea-

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sured; no contrast agent was injected (20). The stability of the catheter position through the entire protocol was verified by comparison of video disc images recorded at the beginning and the end of the procedure. For arterial blood sampling, a short polyethylene catheter or sheath was inserted in the brachial artery in the normal volunteers and the femoral artery in the patients undergoing coronary angiography. The patency of the catheters was maintained by intermittent flushing with 0.9% normal saline (no heparin was used for this purpose).

Specifically labeled [1-$^{14}$C]palmitic acid and [1-$^{14}$C]oleic acid were obtained from New England Nuclear, Boston, MA (50–59 mCi/mmol). Human albumin (25%) was obtained from Cutter Laboratories. The labeled free fatty acid was purchased as its sodium salt or converted to the salt with a small quantity of 0.5 M NaOH. The fatty acid salt was taken up in a small quantity of ethanol, diluted in 0.9% normal saline and bound to an excess of human albumin. The solution obtained was passed through a 0.22-$\mu$m bacteriologic filter (Millipore/Continental Water Systems, Bedford, MA) for sterilization. The [1-$^{14}$C]oleate albumin or [1-$^{14}$C]palmitate albumin was infused intravenously at a constant rate of 25 $\mu$Ci/h.

25–30 min after the start of constant infusion of the tracer, simultaneous coronary sinus and artery samples were obtained for the chemical and radioisotopic analyses of free fatty acids, $^{14}CO_2$ and chemical concentrations of glucose and lactate and hematocrit. In all subjects two to four pairs of samples were obtained at baseline (average time interval, 10 min). To evaluate free fatty acid metabolism over a wide range of circulating levels, heparin (10,000 U as a bolus) was given to 15 subjects. In these subjects, paired samples were obtained 10 and 20 min following heparin administration.

*Chemical analysis.* Blood for free fatty acids was placed in iced, heparinized glass tubes within 30 s of sampling, centrifuged at 4°C, and separated. The free fatty acid content of plasma was determined by a modification of the extraction method of Ko and Royer (22). After extraction of the plasma with heptane/isopropyl alcohol containing pentadecanoic acid as an internal standard, the heptane was evaporated and 0.05 ml of 10% $BCl_3$/methanol (Sigma Chemical Co., St. Louis, MO) was added to convert the fatty acids to the respective methyl esters. After the reaction has proceeded for 10 min, two layers are present: an oily film adhering to the walls of the reaction vial and the $BCl_3$/methanol layer. The latter solution, after transfer to another vial, was evaporated with nitrogen. Methylene chloride (0.025 ml) was added to the vial and a sample was injected into a gas chromatograph using a 10′ × ¼″ ID glass column, 10% SP-2330 (Supelco, Inc., Bellefonte, PA), oven temperature, 190°C. The relative amounts of the individual fatty acids were then determined by comparison with the pentadecanoic acid internal standard and integrated with a 5880 gas chromatograph (Hewlett–Packard Co., Palo Alto, CA). Eight analyses of one sample gave a coefficient of variation of 1.9% in our laboratory. The individual contents of free fatty acids were calculated per milliliter of blood with the hematocrit.

The specific activity of an individual fatty acid was determined as follows: 1.5 ml plasma was extracted with 6.0 ml heptane/isopropyl alcohol as described above, without internal standard. The dried extract was taken up in $CH_2Cl_2$ and streaked onto a thin-layer chromatographic plate (silica gel G, 20 × 20 cm, 250-micron layer; Analtech, Inc., Newark, DE) and developed with a solution of hexane/ether/acetic acid, 82:18:1 by volume. The region of the plate corresponding to free fatty acids was scraped off and extracted with 4.0 ml of 30:70 heptane/isopropyl alcohol and 3 ml of 0.033 M sulfuric acid. The resulting heptane layer was pipetted off and divided into two 0.5-ml portions that were pipetted into a scintillation vial for counting (using 10.0 ml of toluene/Liquofluor [New England Nuclear]) and a small reaction vial, respectively. A known amount of pentadecanoic acid in heptane was pipetted into the reaction vial and the mixture evaporated with $N_2$. The mixture of fatty acids was converted to the respective methyl esters with 0.050 ml of 10% $BCl_3$/methanol, and after 10 min the $BCl_3$/methanol was evaporated and 0.025 ml of $CH_2Cl_2$ added. This mixture was gas chromatographed under the same conditions as described above. The specific activity of the individual fatty acid was calculated with the radioactive content and chemical content of that acid determined by gas chromatographic analysis. The coefficient of variation for this method is 2.5% in our laboratory (eight analyses of one sample). The radioisotopic free fatty acid analyses were all performed in duplicate.

The specific activity of the secondary labeled triglyceride was determined by scraping off the area corresponding to triglycerides of the thin layer chromatographic plate described above. The triglycerides were eluted from the silica in the same fashion as the fatty acids. The heptane layer was then divided into two portions of 0.8 ml, for scintillation counting, and 0.2 ml, for analysis by conversion to the methyl ester as follows. After addition of a known amount of standard triglyceride (Tripalmitin, Sigma Chemical Co.) the heptane was evaporated with $N_2$ and 100 $\mu$l of 2% sodium methoxide (Sigma Chemical Co.) in methanol added to transesterify the triglycerides to their respective methyl esters. The content of the fatty acid of interest was then determined by gas chromatography as described above. The specific activity was then calculated from the dpm content of the 0.8-ml portion and the fatty acid content of the 0.2-ml portion, and is expressed as dpm/nmol of the fatty acid infused.

The amount of interconversion of fatty acids was determined by isolation of the individual fatty acid methyl esters by high pressure liquid chromatography and subsequent scintillation counting. The fatty acids in heptane, after isolation by thin-layer chromatography were converted to their methyl esters with boron trichloride/methanol as described above. The methyl esters were then taken up in 0.1 ml acetone/acetonitrile (1:1 vol/vol) and separated by high-pressure liquid chromatography on three reverse phase 5 micron Supelcosil, LC-18 columns (25 cm × 4.6 mm, Supelco, Inc.) in series using 50:50 acetone/acetonitrile at a flow rate of 2 ml/min. A refractive index detector was used to follow the separation. Each peak was collected in a scintillation vial as it eluted and was subsequently blown dry with $N_2$. The specific activity of each fatty acid was determined by adding a known quantity of methyl pentadecanoate in $CH_2Cl_2$ and performing a gas chromatographic analysis to determine content. The radioactivity present in the vial was then determined by scintillation counting.

The $^{14}CO_2$ was collected directly from the blood by a diffusion method (23). A blood sample was placed in the outer well of a double-chambered Erlenmeyer flask. With the system airtight, lactic acid was added to the blood and the flask agitated on a shaker table for 2 h at room temperature. The center well of the flask contained a known volume of 1.0 M NaOH that trapped the released $^{14}CO_2$ as $NaH^{14}CO_3$. A portion of the $NaH^{14}CO_3$ solution was mixed with Aquasol and counted. The recovery of $^{14}CO_2$ from $NaH^{14}CO_3$ added to whole blood in vitro by this method is 99.5%; in our laboratory the coefficient of variation for this method is 2.9% (eight analyses of one sample).

Blood samples for lactate and glucose were mixed immediately with a measured volume of cold 7% perchloric acid (1:2 vol/vol) and centrifuged. The protein-free supernatant was removed and stored at −4°C for future analysis.

The lactate and glucose concentration were determined in the protein-free fluid by enzymatic methods (24, 25). All chemical analyses were performed in duplicate. To determine incorporation of $^{14}$C label into lactate and glucose, ion exchange chromatography was employed for separation before scintillation counting as previously published (26).

*Calculations.* The chemical substrate extraction or production by the myocardium was calculated as: extraction ratio (%) = ([A] − [CS]/[A]) × 100, where [A] is the arterial concentration of substrate and [CS] is the coronary sinus concentration of substrate. The free fatty acid concentrations were determined in plasma and calculated per milliliter of blood by dividing the plasma level by (1 − hematocrit (%)/100). The glucose, lactate, and $^{14}CO_2$ were determined for content in blood.

For the $^{14}$C-labeled free fatty acids, the extraction ratio was calculated with the specific activity (sp act = dpm/nmol) of the individual free fatty acid as: $^{14}$C extraction ratio (%) = [A] × sp act in A − [CS] × sp act in CS/[A] × sp act in A.

The myocardial palmitate or oleate uptake (nmol/ml) of blood was calculated from the isotope techniques as: $^{14}$C uptake (nmol/ml blood) = [A] × ($^{14}$C extraction ratio/100).

*Table I. Arterial Concentrations of Individual Free Fatty Acids in Plasma*

| Total FFA | Normal subjects (Group I) 382±140 nmol/ml | | Patients with CAD (Group II) 537±163 nmol/ml | |
|---|---|---|---|---|
| | *nmol/ml* | *% of total FFA* | *nmol/ml* | *% of total FFA* |
| Myristic acid (14:0) | 9±4 | 2.3±0.6 | 10±7 | 1.7±0.8 |
| Palmitic acid (16:0) | 97±39 | 25.1±1.8 | 142±53 | 26.2±2.1 |
| Palmitoleic acid (16:1) | 13±8 | 3.3±1.1 | 24±13 | 4.4±1.0 |
| Stearic acid (18:0) | 42±14 | 11.4±2.2 | 55±25 | 10.2±2.1 |
| Oleic acid (18:1) | 152±61 | 39.4±2.8 | 225±63 | 42.2±2.8 |
| Linoleic acid (18:2) | 68±19 | 18.3±2.4 | 79±19 | 15.1±3.0 |
| Linolenic acid (18:3) | 2±2 | 0.5±0.4 | 2±3 | 0.4±0.5 |

All values are expressed as mean±1 SD as nmol/ml of plasma. Individual FFA were measured by gas chromatography. CAD, coronary artery disease; FFA, free fatty acids.

The amount of palmitate or oleate oxidized was calculated from the myocardial production of $^{14}CO_2$ as: amount oxidized (nmol/ml blood) = ($^{14}CO_2$ (CS − A) dpm/ml)/sp act of the individual FFA.

*Statistical analysis.* Linear regression analyses were performed to compare various metabolic parameters with the circulating levels of the individual free fatty acids. The standard errors of the estimate were calculated (27). The two-tailed unpaired *t* test was used to compare the metabolic parameters between the two groups of subjects. The two-tailed paired *t* test was used to compare two values in the same subjects. The data are presented as mean±1 SD.

## Results

24 subjects were included in the study; 13 were young healthy male volunteers (group 1) with mean age of 22±3 yr and 11 subjects had undergone coronary angiography for symptoms of ischemic heart disease (group II) with mean age of 52±13 yr. Six patients in group II had significant triple vessel coronary disease; three of these six had significant lesions in the left main coronary artery. Two of the remaining five had significant lesions in two coronary vessels; one had a significant lesion in only one coronary vessel and two subjects had no significant coronary lesions. A significant lesion was defined as that compromising the luminal diameter by 50% or greater. The mean left ventricular ejection fraction was 66±12% in group II; no patient had an ejection fraction of < 45%. During the metabolic sampling periods, the heart rate was 66±9 beats per minute and the mean arterial pressure was 89±6 mmHg in group I. These values in group II were 59±14 beats per minute and 101±17 mmHg, respectively.

The baseline (preheparin) arterial free fatty acid level in the young normal subjects (group I) was 382±140 nmol/ml of plasma as measured by gas chromatography. In the ischemic heart disease patients (group II) the level was 537±163 nmol/ml of plasma. The baseline arterial levels of the individual free fatty acids and their percent of the total free fatty acids are given in Table I for both subject groups. The oleate level was 152±61 nmol/ml of plasma in the normal subjects and 225±63 nmol/ml of plasma in the ischemic heart disease patients. The palmitate level was 97±39 nmol/ml and 142±53 nmol/ml, respectively. Although the circulating levels of the free fatty acids are higher in group II compared with group I ($P < 0.025$), the relative percentage of each free fatty acid was similar for both groups.

To present our results for free fatty acid oxidation, calculated from the myocardial production of $^{14}CO_2$ per milliliter of blood, the free fatty acid levels in nmol/ml of plasma were converted to nmol/ml of blood by using the hematocrit. Thus, all further results in this study will be expressed per milliliter of blood. The baseline arterial level of free fatty acids per milliliter of blood was 223±83 nmol in group I compared with 382±140 nmol/ml of plasma, and for group II these values were 310±95 nmol and 537±163 nmol, respectively. Table II shows the circulating levels of free fatty acids, glucose, and lactate and the arterial-coronary sinus chemical differences per milliliter of blood for these substrates before heparin administration. In the 15 subjects receiving heparin, the circulating level of free fatty acids rose from a baseline of 255±96 nmol/ml of blood to 455±142 nmol/ml following the heparin ($P < 0.001$).

In group I, 6 normal subjects received [1-$^{14}$C]palmitate albumin for the isotope infusion and 7 subjects received [1-$^{14}$C]oleate albumin. Three of the patients in group II had [1-$^{14}$C]palmitate albumin while eight had [1-$^{14}$C]oleate albumin.

At a constant infusion of $^{14}$C-labeled free fatty acids of 25 μCi/h, 25–30 min of infusion are required to achieve a steady state of the specific activity and myocardial production of $^{14}CO_2$. In this study the time from the initiation of the constant isotope infusion to the first metabolic samples was 42±14 min. In the subjects receiving [1-$^{14}$C]palmitate the specific activity of arterial palmitate was 9.28±4.01 dpm/nmol and the coronary sinus–arterial $^{14}CO_2$ difference was 259±52 dpm/ml. In the [1-$^{14}$C]oleate subjects, the specific activity of arterial oleate was 4.67±2.81 dpm/nmol and the $^{14}CO_2$ difference was 245±124 dpm/ml. Fig. 1 shows the arterial and coronary sinus specific

*Table II. Arterial Substrates and Myocardial Chemical Substrate Uptake*

| | Arterial FFA | A-CS FFA | Arterial glucose | A-CS glucose | Arterial lactate | A-CS lactate |
|---|---|---|---|---|---|---|
| | *nmol/ml** | *nmol/ml* | *μmol/ml* | *μmol/ml* | *μmol/ml* | *μmol/ml* |
| Normal subjects (Group I) | 223±83 | 95±35 | 4.94±0.41 | 0.19±0.14 | 0.589±0.135 | 0.185±0.103 |
| Patients with CAD (Group II) | 310±95 | 107±23 | 5.38±0.53 | 0.11±0.16 | 0.665±0.211 | 0.132±0.092 |

* All values expressed as mean±1 SD per ml of blood. All values are before heparin. A, arterial; CS, coronary sinus; CAD, coronary artery disease; FFA, free fatty acids.

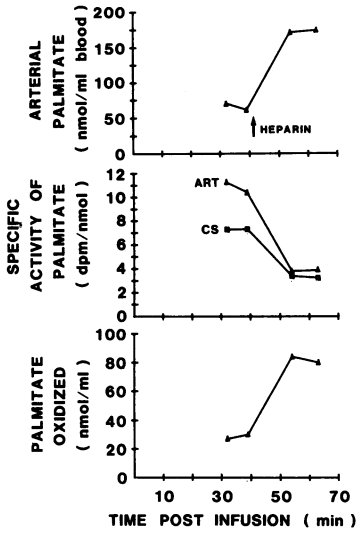


*Figure 1.* The arterial level of palmitate and the arterial and coronary sinus specific activities of palmitate are shown over time in a subject receiving [1-$^{14}$C]palmitate. The constant infusion of [1-$^{14}$C]palmitate at 25 $\mu$Ci/h was started at time 0. The amount of palmitate oxidized by the myocardium is also shown for the same subject. Heparin (10,000 U) was given intravenously at 42 min.

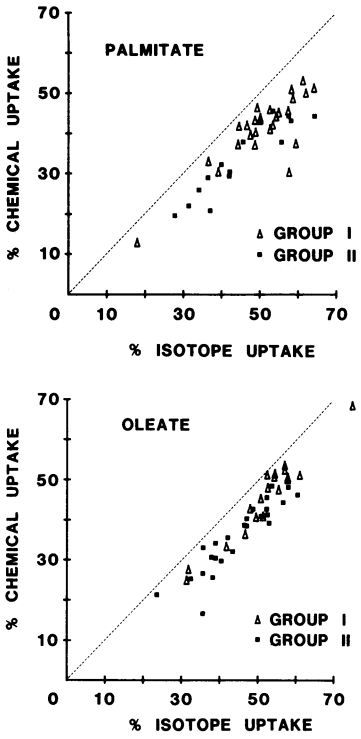


*Figure 2.* Comparison of the chemical and isotope extraction ratios of palmitate (*upper*) in all subjects receiving [1-$^{14}$C]palmitate and oleate (*lower*) in all subjects receiving [1-$^{14}$C]oleate. (*Dashed line*) is the line of identity for the chemical and isotope extraction ratios.

activity, the amount of palmitate oxidized and the arterial level of palmitate in a subject over the time course of the metabolic study.

When $^{14}CO_2$ is used to assess substrate oxidation, the contribution of $^{14}$C from oxidation of other metabolic substates that might become labeled with the isotope secondarily must be considered. After 65 min of [1-$^{14}$C]palmitate or [1-$^{14}$C]oleate infusion at 25 $\mu$Ci/h, the specific activities of glucose and lactate in 12 subjects were 0.005±0.002 dpm/nmol and 0.034±0.024 dpm/nmol, respectively, compared with 4.71±1.36 dpm/nmol for the individual labeled free fatty acid. Likewise the specific activity of the fatty acids of the triglycerides was 0.40±0.27 dpm/nmol compared with 8.81±5.55 dpm/nmol of the free fatty acid being studied. The secondary labeling between free fatty acids was measured with high pressure liquid chromatography. In the last metabolic sample obtained, < 2% of the $^{14}$C label was found on other free fatty acids.

*Chemical extraction vs. isotope extraction.* Fig. 2 compares the traditional arterial-coronary sinus chemical extraction ratio with the $^{14}$C-isotope extraction ratio. If the chemical extraction ratio were equal to the isotope extraction ratio, the points would fall on the line of identity. In all subjects both before and after heparin, the isotope extraction ratio was greater than the chemical ratio. For [$^{14}$C]palmitate the isotope extraction ratio was 52±9% compared with a chemical ratio of 40±9% ($P < 0.001$). Similarly for [$^{14}$C]oleate the ratios were 48±10% and 40±10%, respectively ($P < 0.001$). These data imply that the traditional chemical arterial-coronary sinus difference underestimates the actual uptake of free fatty acids.

Arterial-coronary sinus chemical uptakes and the isotope uptakes are shown in Tables III and IV for the two subject groups; all values were obtained before heparin. For palmitate and oleate the differences between the chemical and isotope uptakes were 13±5 and 12±4 nmol/ml, respectively, in group II. These values were significantly greater than those in group I, 5±1 and 7±3 nmol/ml ($P < 0.005$ and $P < 0.05$, respectively). Thus in young normal subjects (group I) the actual uptake of palmitate or oleate was underestimated by 16±8% as compared with 23±9% in the ischemic heart disease patients (group II) ($P < 0.001$) before heparin.

After heparin there was a significant increase in the differences between the isotope and chemical uptakes for the group II coronary artery disease patients. Before heparin this value was 12±4 nmol/ml and increased with heparin to 19±6 nmol/ml ($P < 0.005$). In the group I normal volunteers this difference was 5±1 nmol/ml before heparin and 7±2 nmol/ml following heparin; this change was not statistically significant.

*Free fatty acid oxidation.* Oxidation of palmitate and oleate was calculated by measuring the myocardial production of $^{14}CO_2$ and the specific activity of the individual free fatty acid. The $^{14}$C-isotope uptake and the amount oxidized of the individual free fatty acids are shown in Tables III and IV for both subject

*Table III. Myocardial Metabolic Fate of Palmitate*

| | Arterial palmitate | A-CS palmitate | [$^{14}$C]Palmitate uptake | Palmitate oxidized | Difference $^{14}$C and A-CS uptake |
|---|---|---|---|---|---|
| | *nmol/ml* | *nmol/ml* | *nmol/ml* | *nmol/ml* | *nmol/ml* |
| Normal subjects (Group I) | 47±8 | 21±6 | 26±6 | 22±6 | 5±1 |
| Patients with CAD (Group II) | 91±45 | 28±2 | 42±6 | 35±10 | 13±5 |

Results expressed as mean±1 SD as nmol/ml of blood. All values are before heparin. A, arterial; CS, coronary sinus; CAD, coronary artery disease.

*Table IV. Myocardial Metabolic Fate of Oleate*

| | Arterial oleate | A-CS oleate | [$^{14}$C]Oleate uptake | Oleate oxidized | Difference $^{14}$C and A-CS uptake |
|---|---|---|---|---|---|
| | *nmol/ml* | *nmol/ml* | *nmol/ml* | *nmol/ml* | *nmol/ml* |
| Normal subjects (Group I) | 121±27 | 55±12 | 61±11 | 52±11 | 7±3 |
| Patients with CAD (Group II) | 130±40 | 51±11 | 62±14 | 45±13 | 12±4 |

A, arterial; CS, coronary sinus; CAD, coronary artery disease.

groups. Fig. 3 shows the percentage of the $^{14}$C uptake that underwent rapid oxidation for each data point. In the subjects receiving [$^{14}$C]palmitate, 86±15% of the palmitate extracted underwent rapid oxidation in group I; for group II the percentage was similar (86±21%).

In the subjects receiving [$^{14}$C]oleate, the amount of oleate extracted was significantly higher compared with palmitate ($P < 0.001$). Despite this difference, the percentages undergoing rapid oxidation were similar: 86±16% for group I and 78±17% for group II.

After heparin administration, there was an increase in the amount of palmitate and oleate oxidized in both subject groups, 24±6 nmol/ml before heparin vs. 49±24 nmol/ml following heparin for palmitate and 46±13 nmol/ml vs. 68±15 nmol/ml for oleate, respectively. However there was no significant change in the percentage being oxidized, 80±6% preheparin and 86±20% postheparin for palmitate and 75±14% and 85±11%, respectively, for oleate.

*Arterial levels of free fatty acids.* The baseline levels of arterial palmitate and oleate are given in Tables III and IV. Heparin was given to elevate the circulating levels in 15 subjects. The range of arterial palmitate was 32–176 nmol/ml of blood in the subjects receiving [$^{14}$C]palmitate. Fig. 4 demonstrates a significant correlation between the arterial level of palmitate and the amount being oxidized by the myocardium in these subjects ($r = 0.82$; $P < 0.001$).

The range of circulating oleate in the subjects receiving [$^{14}$C]oleate was 82–272 nmol/ml of blood. A similar significant positive correlation was present between the arterial level and amount oxidized ($r = 0.77$; $P < 0.001$) (Fig. 5).

In the young normal subjects (group I) a significant inverse correlation was present between the arterial level of free fatty acids and the arterial-coronary sinus chemical difference for lactate ($r = -0.62$; $P < 0.001$). Likewise for myocardial glucose extraction, a negative correlation was observed between the circulating level of free fatty acids and the arterial-coronary sinus glucose difference ($r = -0.56$; $P < 0.001$).

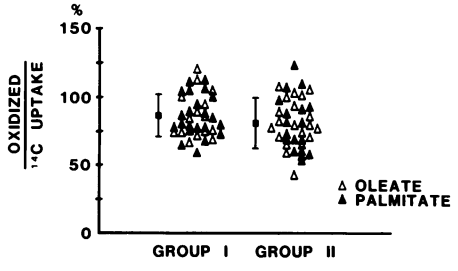


*Figure 3.* The percent of the $^{14}$C-isotope uptake of palmitate or oleate that underwent rapid oxidation by the myocardium. The data points in the young normal subjects in group I (*left column*) and in the ischemic heart disease patients in group II (*right column*) are shown and the mean±1 SD for the two subject groups are represented.

## Discussion

The importance of free fatty acids as a substrate for myocardial metabolism is well recognized (3–15, 28–30). This study demonstrates for the first time in humans that a high percentage (84±17%) of the free fatty acids extracted undergoes rapid oxidation. Isotopically labeled palmitate and oleate with $^{14}$C were used as tracers. These free fatty acids were selected because they comprise 65% of the total circulating free fatty acids in humans. Free fatty acid oxidation was calculated from the myocardial production of $^{14}CO_2$ and the specific activity of the individual free fatty acid labeled with $^{14}$C as determined by thin layer chromatography/gas chromatography.

Other investigators using similar tracers have found that a significantly lower percentage from 30–60% of the free fatty acid extracted was oxidized in humans (31–33). Miller et al. (34) reported that 100% of the free fatty acids extracted by the myocardium underwent oxidation in unanesthetized dogs. In the previous human studies the isotope infusion times varied from 8–30 min. Many of the metabolic samples were obtained after only 15 min of infusion, and the data showed that the myocardial production of $^{14}CO_2$ had not achieved a steady state. In the present study, the average time from the initiation of the constant isotope infusion to the first metabolic samples was 42 min with a range of 22–73 min. Miller and colleagues' first metabolic sample was obtained 60 min after the start of the isotope infusion.

In addition, in previous studies the free fatty acid content was measured by either a titration or a $^{63}$Ni complex method. Both these methods measure short-chain fatty acids and other acids as well as the long-chain fatty acids and give values 100–200 nmol/ml of plasma higher than gas chromatography. The specific activity of the free fatty acids was measured by scintillation counting the appropriate portion of the thin-layer chromatographic plate while assuming a standard measured recovery, or by scintillation counting the heptane–alcohol layers obtained in the titration technique. In the latter method the free fatty

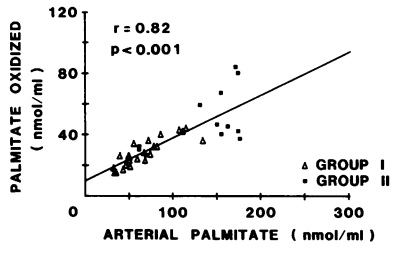


*Figure 4.* Correlation between arterial blood palmitate levels and the myocardial oxidation of palmitate in the six normal subjects (group I) and three ischemic heart disease patients (group II) receiving [1-$^{14}$C]palmitate. $n = 36$, $Y = 282\ X + 9.7$; standard error of estimate 9.6 nmol/ml.

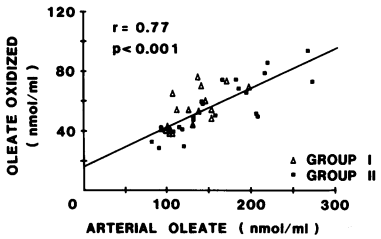


*Figure 5.* Correlation between arterial blood oleate levels and the myocardial oxidation of oleate in the seven normal subjects (group I) and eight ischemic heart disease patients (group II) receiving [1-$^{14}$C]oleate. $n$ = 41, Y = 261 X + 16.1; standard error of estimate 10.3 nmol/ml.

acids were not separated from the other lipids; thus this methodology could lead to specific activities that were falsely high. In both methods, however, the specific activity calculation may be inaccurate, which would lead to errors in determining the amount of free fatty acids oxidized. In our study the free fatty acid portion of the thin-layer chromatographic plate was counted for $^{14}$C and also analyzed for free fatty acid content by gas chromatography to determine specific activity. We believe that the duration of our isotope infusion that allowed a steady state to be achieved and the use of thin-layer chromatography/gas chromatography to determine the specific activity of the individual free fatty acids resulted in our finding that a greater portion of the free fatty acid extracted underwent oxidation in human subjects.

Using the techniques described, we can measure the myocardial production of $^{14}CO_2$ and quantitate free fatty acid oxidation; however we are not able to determine if the free fatty acid enters the oxidative pathway in the mitochondria directly or if it is initially incorporated into a triglyceride pool with a rapid turnover rate (35). Thus, in this report we have referred to the palmitate and oleate oxidation as "rapid" as opposed to "immediate" or "direct."

The myocardial isotope extraction ratio for free fatty acids has been reported to be greater than the chemical extraction ratio by several investigators in humans (31–33, 36), animals (37) and perfused heart experiments (38). This is the first study to demonstrate that this difference was greater in older patients with ischemic heart disease than in young normal male subjects. The isotope extraction ratio is greater than the chemical ratio because unlabeled free fatty acids (palmitate or oleate) are appearing in the venous effluent of the myocardium. Therefore the chemical extraction ratio underestimates the actual uptake of free fatty acids by the myocardium.

There are three possible etiologies for this appearance of unlabeled free fatty acids. Myocardial cells have stores of triglycerides. During ischemia, triglycerides have been shown to increase (39). The 11 older subjects in group II had symptoms of ischemic heart disease, and nine of the 11 had significant coronary artery lesions. However, there was no evidence of acute ischemia during the metabolic procedure. None of these subjects had unstable angina, chest pain, or electrocardiographic changes during the metabolic sampling. Our finding of a high percentage of free fatty acid being oxidized and that the percentage was similar to the value in the young normal subjects would suggest that ischemia was not present. However, chronic intermittent ischemia over a period of time may have induced alterations in the activity of metabolic pathways (40) or in substrate pools such as triglycerides. The time required for the biochemical changes induced by an ischemic episode to return to baseline or normal in the human myocardium is not known (41, 42). Alteration in substrate pools or turnover rates induced by chronic intermittent ischemia may account for the difference between the isotope and chemical free fatty acid uptakes found in the two subject groups.

Fox and his colleagues (43) investigated the metabolic fate of [1-$^{11}$C]palmitate in open-chest anesthetized dogs. Under nonischemic conditions they reported that 6±3% of the tracer extracted by the myocardium back-diffused within 10 min, i.e., it was released nonmetabolized from the myocardium. Under conditions of ischemia, they found a higher percentage (16±9%) was back-diffused. If indeed back-diffusion or release of free fatty acids from the myocardial cells is occurring, the continuous isotope infusion method used in this investigation would tend to underestimate the amount because the $^{14}$C label would also back-diffuse as it equilibrated with intracellular pools.

A second source for the unlabeled free fatty acids might be the lipids in the myocardial interstitial spaces. In a morphological study, Roy (44) demonstrated that lipid droplets are present in the myocardial interstitial spaces in dog and man. Julien et al. (37) showed that this lipid pool had a very slow turnover of free fatty acids. Pathologic studies have shown that with aging there is an increase in this interstitial lipid accumulation (45). The mean±SD age of the subjects in group II with ischemic heart disease symptoms was 52±13 yr, significantly greater than the mean age of 22±3 yr in the normal subjects in group I. Thus, this lipid pool in the myocardial interstitial space may be the source of the unlabeled free fatty acids that appeared in the venous effluent. The differences observed between the two subject groups may be related to aging.

The third possible explanation for the differences in the unlabeled free fatty acids in the venous effluent is hydrolysis of circulating triglycerides. Muir (46) has shown that lipoprotein lipase exists in the myocardium. Hydrolysis of circulating triglycerides results in the release of fatty acids. If these fatty acids are not immediately extracted by the myocardium, they may be bound to circulating albumin and thus appear in the venous effluent as unlabeled free fatty acids. The baseline triglycerides were 60±29 mg/dl in the normal subjects and were significantly higher, 264±70 mg/dl in the ischemic heart disease patients. Thus, hydrolysis of circulating triglycerides could also account for our finding a significant difference in the unlabeled free fatty acids between these two subject groups before heparin. Heparin increases lipoprotein lipase activity (47). After heparin administration, there was a significant increase in the difference between the isotope and chemical uptakes in the subjects in group II of our study. This increase in unlabeled free fatty acids with heparin administration indicates that it is related to hydrolysis of circulating triglycerides and suggests that this finding in the preheparin samples may also be related to circulating triglycerides.

Evans and his coworkers (38) investigated the uptake and oxidation of palmitate in an isolated perfused rat heart. The perfusion fluid was a modified Krebs–Henseleit bicarbonate buffer containing albumin-bound fatty acid with [$^{14}$C]palmitate as a tracer. They reported a similar discrepancy between the isotope and chemical uptake. This finding in an isolated rat heart being perfused without triglycerides in the media suggests that the unlabeled free fatty acids are being released from the myocardial cells or interstitial lipid stores and not from the hydrolysis of circulating triglycerides. Thus, we believe that further investigation is necessary to demonstrate the precise etiology for the appearance of unlabeled free fatty acids in the venous effluent observed in this and other studies (31–33, 36–38).

It has long been recognized that the arterial level of substrate is very important in determining the myocardial uptake of the substrate (4, 6, 28, 36, 48). This has been well demonstrated for free fatty acids. Rothlin and Bing (49) measured the myocardial chemical extraction ratios of the individual free fatty acids and found that the myocardial uptake of each fatty acid differed. The myocardial uptake of the individual fatty acids was related to their level in the plasma. In the present study we also found a highly significant positive correlation between the arterial levels of palmitate and oleate and the amount of these free fatty acids oxidized by the myocardium (Figs. 4 and 5). Thus, it appears that the circulating levels of free fatty acids are not only important in determining the myocardial uptake but also are an important determinant in the amount being oxidized.

Lassers et al. (50) have found a negative correlation between the arterial free fatty acid level and the myocardial glucose and lactate uptake. We have also demonstrated similar findings for lactate in normal subjects (51). Likewise in this present study, a significant negative correlation was observed between the arterial free fatty acid level and myocardial lactate ($r = -0.62$) and glucose ($r = -0.56$) chemical uptake in the normal subjects. Using [6-$^{14}$C]glucose and measuring glucose oxidation, we recently reported a significant inverse correlation between circulating free fatty acids and myocardial glucose oxidation (52). Randle and his colleagues (53–56) have shown that free fatty acids affect glucose uptake and oxidation at several enzymatic steps in isolated rat hearts. If the level of circulating free fatty acids is high, our data support that the myocardium preferentially oxidizes free fatty acids. When this occurs, the levels of ATP and acetyl-coenzyme A are high. High levels of acetyl-coenzyme A inhibit the enzyme pyruvate dehydrogenase, which is involved in the oxidative decarboxylation of pyruvate. Inhibition of this enzyme blocks the entry of glucose and lactate into the citric acid cycle. In addition, when the myocardium is actively utilizing free fatty acids, there is a build-up of citrate. Citrate inhibits the enzyme phosphofructokinase in the glycolytic pathway. When this latter enzyme is inhibited, glucose-6-phosphate accumulates in the cell. As this occurs, further phosphorylation of glucose and finally glucose uptake into the cell are inhibited. Our data in humans in a resting, fasting state support the concept that the circulating levels of free fatty acids are very important in regulating myocardial utilization of free fatty acids as well as glucose oxidation and the myocardial uptake of glucose and lactate.

In summary, this study demonstrates that a high percentage of the free fatty acids extracted by the myocardium undergoes rapid oxidation in humans. A highly significant positive correlation was present between the arterial level of the individual free fatty acid and the amount being oxidized. In addition, the arterial-coronary sinus difference underestimated the actual myocardial extraction of free fatty acids; this difference was significantly greater in the older patients with ischemic heart disease than in the young normal subjects.

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