Utilization of Long-Chain Free Fatty Acids by Human Platelets

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ABSTRACT There was a rapid net uptake of free fatty acid (FFA) by human platelets when long-chain FFA, bound to human serum albumin, were incubated with platelet suspensions. Results from experiments in which both palmitate and albumin were labeled indicated that the fatty acid dissociated from the protein during uptake. Much of the FFA taken up by the platelet in short-term incubations remained in unesterified form, i.e., it was recovered as platelet FFA. As the incubation continued, increasing amounts of FFA were oxidized to CO_2 and incorporated into platelet lipid esters, particularly lecithin. Essentially all of the fatty acid that was incorporated into the platelet FFA fraction was released rapidly from the cells when they were exposed to a medium containing FFA-free albumin. The magnitude of uptake into the platelet FFA fraction was similiar at 0° and 37°C. Likewise, the rate and magnitude of FFA release from the platelet were similar at 0° and 37°C. Therefore, it is likely that both FFA uptake and FFA release occur by energy-independent mechanisms. The major effect of increasing the FFA concentration of the incubation medium was increased fatty acid uptake into the platelet FFA fraction. Similar results occurred when platelets were incubated in human plasma containing increasing amounts of added palmitate. At a given extracellular FFA concentration, considerably more of the saturated fatty acids, palmitate and stearate, were taken up as platelet FFA than either oleate or linoleate.

INTRODUCTION

Palmitic, stearic, oleic, and linoleic acids account for approximately 85% of the long-chain free fatty acid (FFA) present in human plasma (1). Intravenous infusions of isotopic tracers in humans have shown that the rates of utilization of these FFA are, in general, similar (2, 3). On the other hand, detailed comparative studies with mammalian cell suspensions have demonstrated differences in the extent to which each fatty acid is taken up and metabolized (4, 5). For example, much more stearate than linoleate was taken up as FFA by erythrocytes and Ehrlich ascites tumor cells (4, 5). In contrast, more linoleate than stearate was incorporated into lipid esters in both of these cells (4, 5).

Human platelets, like most other mammalian cells, take up and metabolize long-chain FFA (6–8). Palmitic acid has been employed in most studies of FFA metabolism in platelets (6, 7), and except for incorporated into phospholipids (8), little is known about the way in which the other commonly occurring long-chain fatty acids are utilized. This question is of particular relevance for platelets because it has been reported that saturated FFA are much more effective than unsaturated FFA in causing platelet aggregation (9, 10). Such effects may result from differences in the way that platelets take up or metabolize saturated and unsaturated FFA. In order to examine this point, we compared the utilization of palmitic, stearic, oleic, and linoleic acids by suspensions of human platelets. The major difference that we observed was the extent to which each of these fatty acids was incorporated in unesterified form by the platelet. At a given extracellular FFA concentration, much more palmitate and stearate, the saturated fatty acids, were incorporated into the platelet FFA fraction than either oleate or linoleate.

METHODS

Preparation of platelets. Venous blood was taken from normal, nonfasting human donors. For most experiments 460 ml of blood was collected in 38 ml of 0.077 M disodium ethylenediaminetetraacetate (EDTA) adjusted to pH 7.4. The freshly collected blood was added to siliconized tubes and centrifuged for 15 min at 300 *g*. The platelet-rich plasma was taken off and recentrifuged at 750 *g* for 15 min

Preliminary reports of portions of this work were presented at the 4th Conference on Platelets, Oak Ridge, Tenn., 23 June 1969, and to the Council on Arteriosclerosis, Dallas, Tex., 13 November 1969.

Received for publication 22 December 1969 and in revised form 24 February 1970.

in order to obtain the platelet pellet. After removing the plasma, the platelets were resuspended in a solution containing 0.123 M NaCl, 0.0045 M KCl, and 0.015 M Tris buffer adjusted to pH 7.4 with 1 N HCl. The platelet count of each suspension also was determined, and enough Tris-buffered salt solution was added so that 1 ml of the suspension contained 10^9 platelets. Microscopic examination of the platelet suspension revealed only trace contamination with other cellular components of the blood. White blood cells were the major contaminant. However, they usually made up approximately 0.07% of the cellular elements of the platelet suspension, and they never exceeded a concentration of 0.16%. In all experiments incubation of the platelet suspension was begun from 1 to 1.5 hr after the blood was drawn from the donor.

When blood plasma was required, the supernatant solution obtained after sedimentation of the platelets from platelet-rich plasma was recentrifuged as above, and then stored in an ice bath until the platelets were ready for incubation.

Preparation of the FFA-albumin solution. Unlabeled fatty acids were purchased from the Hormel Institute. Oleic acid-1-^{14}C and linoleic acid-1-^{14}C were obtained from Nuclear-Chicago Corp. Palmitic acid-1-^{14}C and stearic acid-1-^{14}C were supplied by New England Nuclear. Each radioactive long-chain fatty acid was dissolved in *n*-heptane and extracted into alkaline ethanol. The ethanol phase was removed and acidified, and the labeled fatty acids were then reextracted into fresh heptane (11). Thin-layer chromatography on Silica Gel G of aliquots of each labeled fatty acid solution indicated that at least 98% of the radioactivity applied to the chromatogram was recovered as FFA. Analyses by gas-liquid chromatography revealed that the radiopurity of these fatty acids was between 94 and 99% (11).

Crystalline human serum albumin was obtained from Pentex Biochemical. The protein was incubated with charcoal according to the method of Chen in order to remove bound fatty acids (12). After neutralization with NaOH, 100 ml of the protein solution was dialyzed overnight at 4°C against 4 liters of distilled water, the water being changed at least once during the period of dialysis (11). Tris buffer, KCl, and NaCl were added, and the albumin was adjusted to pH 7.4 with 0.1 N HCl. The final solution contained 0.123 M NaCl, 0.0045 M KCl, 0.015 M Tris buffer, and 0.1 μmole/ml albumin, pH 7.4.

FFA-albumin solutions were prepared by incubating aliquots of the Tris-buffered albumin solution with FFA-coated Celite (13). A mixture of radioactive and carrier FFA was dissolved in hexane and added to Celite that had been washed to remove acid-soluble materials (obtained from Johns-Manville Products Corp.), and the solvent was evaporated under nitrogen. Weighed quantities of FFA-coated Celite were incubated for 30 min at room temperature with Tris-buffered albumin solution. The suspension was stirred slowly with a magnetic bar. Celite was removed by centrifugation at 0°C for 10 min at 10,000 *g*. The supernatant solution was recentrifuged as above, and the solution was passed through a Millipore filter of pore size 1.2 μ in order to insure complete removal of the Celite. Aliquots of the FFA-albumin solution were extracted with isopropyl alcohol–heptane–1 N H_2SO_4 (40:10:1) and the heptane phase isolated in order to determine the FFA content of each preparation (14). Titration of the heptane phase with 0.02 M NaOH was performed using Nile blue A solution as the indicator (14, 15). Other aliquots of the FFA-albumin solution (200 μl) were added to liquid scintillation counting vials containing 18 ml of scintillator solution made up of 0.3% 2,5-diphenyloxazole and 0.01% 1,4-bis-2-(4-methyl-5-phenyloxazole)benzene in toluene and methanol (70:30, v/v) (11). All liquid scintillation counting was done in a Packard Tri-Carb 3380 spectrometer, and quenching was monitored with the external standard. The specific radioactivity of the FFA in each preparation was calculated from these values. The protein content of the FFA-albumin solution was determined by the biuret method (16), and the molar ratio of each FFA-albumin solution also was calculated.[1]

Incubation and analyses. Platelets were incubated with the various radioactive FFA-albumin solutions. In most experiments, 1 ml of the platelet suspension containing 10^9 cells was incubated with 2 ml of FFA-albumin solution at 37°C with air as the gas phase. These samples were placed in a metabolic incubator and shaken at a rate of 90 strokes per minute. The incubation was terminated by pouring the contents of the flask into 10 ml of cold Tris-buffered salt solution containing 0.0015 M EDTA and rapidly sedimenting the platelets by centrifugation at 0°C for 5 min at 5500 *g*. After the supernatant solution was siphoned off, the platelet pellet was resuspended in 10 ml of fresh buffer and resedimented as above. Washing and resedimentation was repeated three times. The final platelet pellet was extracted with 20 ml of chloroform-methanol solution (2:1, v/v). Extraction was allowed to proceed for at least 1 hr at room temperature, and the samples were shaken vigorously at frequent intervals during this period. The extraction mixtures were transferred to 60-ml separatory funnels, and the chloroform phase was isolated following addition of 5 ml of 0.04 N HCl (14). After the chloroform phase was taken off and dried under a stream of nitrogen, the lipid extract was transferred to a liquid scintillation counting vial and dried under a stream of nitrogen. Toluene-methanol scintillator solution was added, and the radioactivity contained in the total lipid extract was determined in the liquid scintillation spectrometer. Additional aliquots of the platelet lipid extract were chromatographed on thin layers of Silica Gel G. Neutral lipids were separated from phospholipids with a solvent system containing hexane–diethyl ether–methanol–acetic acid (90:20:2:3) (14). The individual phospholipids were separated in a two dimensional chromatography system: first dimension, chloroform–methanol–ammonium hydroxide–water (100:70:6:6); second dimension, chloroform–methanol–acetic acid–water (160:50:2:6) (17). Each chromatogram was exposed to iodine vapor in order to visualize and outline those areas of the silica gel containing lipids. In the one dimensional separation, standards obtained from Applied Science Labs, Inc., were applied to the same chromatogram as the test sample. The two dimensional system was standardized on separate chromatograms with phospholipid standards also obtained from Applied Science. Palmityl carnitine was synthesized from *dl*-carnitine and palymityl chloride (18) and was included as a standard in the system for phospholipid separation. After the iodine had sublimed, the outlined areas of silica gel were scraped directly into liquid scintillation

[1] The molar ratio is an approximation obtained by dividing the total FFA concentration by the total albumin concentration, and therefore does not take account of the fact that some FFA is present in free solution, i.e., not actually bound to albumin. From the association constants for the FFA-albumin complex, it is calculated that more than 99% of the FFA present in media used for these experiments is bound to albumin (11). Therefore, the error introduced by approximating the molar ratio is negligible.

counting vials containing 15 ml of the dioxane-water scintillator solution described by Snyder (19). The radioactivity present in each vial was measured in the liquid scintillation spectrometer and was expressed as a fraction of the total radioactivity recovered from the chromatogram.

Oxidation of labeled exogenous FFA to $^{14}CO_2$ was measured in additional aliquots of the platelet suspension. Incubation vials containing removable center wells were employed (14). The center wells contained 0.2 ml of 1 N KOH and a small filter paper wick. At the end of the incubation period 0.3 ml of 6 N H_2SO_4 was injected through the rubber serum stopper and the flask was shaken at 37°C for 60 min. After chilling briefly in ice, the serum stoppers were removed and the center wells were added to liquid scintillation solution (14).

In additional experiments platelets were loaded with fatty acid by a brief exposure to a medium containing labeled FFA-albumin, washed thoroughly with buffer, and then reincubated in a "FFA-free" albumin-containing medium. Platelets were exposed for 2 min to a labeled FFA-albumin solution and washed three times with Tris-buffered salt solution as described above. The resulting platelet pellet was resuspended in fresh buffer solution so that the platelet concentration was approximataely 10^9/ml. Several aliquots of the platelet suspension were added directly to 20 ml of chloroform-methanol solution in order to determine the platelet lipid radioactivity and distribution before the second incubation. Other aliquots of the labeled platelet suspension were incubated for varying periods of time in media containing FFA-extracted albumin. After incubation the contents of each flask were transferred to a chilled, dry centrifuge tube, and the platelets were sedimented at 0°C for 5 min at 4000 *g*. Aliquots of the supernatant solution were added to 20 volumes of chloroform-methanol solution, and the radioactivity present in the extracted chloroform phase was measured. In some experiments aliquots of the chloroform phase were subjected to thin-layer chromatography in order to determine the distribution of the lipid radioactivity that was released.

Comparison of FFA and albumin uptake. ^{131}I-labeled human serum albumin was a gift from Dr. Frank Cheng of the Nuclear Medicine Section. Palmitic acid-1-^{14}C was added to the ^{131}I-labeled albumin dissolved in Tris-buffered salt solution as described above. Platelets were incubated in this medium for 5 min and then sedimented and washed three times. The platelet pellet was taken up in 2.5 ml of fresh buffer solution. A 1 ml aliquot of the platelet suspension was added to a counting vial and the ^{131}I radioactivity measured in a well-scintillation spectrometer. Another 1 ml aliquot of the platelet suspension was extracted with chloroform-methanol solution, and the ^{14}C content of the isolated chloroform phase was measured in the liquid scintillation spectrometer. The ^{14}C and ^{131}I content of the medium before exposure to the platelet suspension also was determined. In additional experiments, both palmitate-1-^{14}C and albumin-^{131}I were added to plasma, and the labeled plasma was incubated with platelets for 5 min. The ^{14}C and ^{131}I contents of the plasma before incubation and of the platelets after incubation and washing was determined.

Distinction between isotopic exchange and net FFA uptake. In order to determine whether the uptake of radioactive FFA results from a net transfer of fatty acid to the platelets or exchange with unlabeled FFA already contained in the platelet, the following series of experiments was performed. Platelets were incubated for 2 min at 37°C with palmitate-1-^{14}C–albumin and then sedimented from the medium as described above. 1 ml of the supernatant solution was extracted with 5 ml of isopropanol–heptane–1 N H_2SO_4, and the heptane phase was isolated. The ^{14}C content of 0.5 ml of heptane was determined by liquid scintillation techniques. In another aliquot of the heptane extract, the long-chain FFA content was measured colorimetrically by the method of Mahadevan, Dillard, and Tappel (20). Similar analyses of the medium were done before incubation with platelets, and corrections were made for the volume of buffer solution contained in the added platelet suspension. Net uptake of both radioactivity and FFA by the platelets was calculated from the decrease of the content of these substances in the incubation medium. From these values the specific radioactivity of the medium FFA before and after exposure to platelets was determined.

RESULTS

Kinetics of utilization. The utilization of palmitate-1-^{14}C during the course of a 1 hr incubation with isolated human platelets is illustrated in Fig. 1. Palmitate-1-^{14}C was rapidly taken up by the platelets from the albumin-containing incubation medium. At the early time points, most of the labeled palmitate uptake was present in unesterified form, i.e., as platelet FFA. For example, after 2 min of incubation, 14% of the radioactivity taken up was present in phospholipids, 2% in glycerides and 84% as FFA. As the incubation proceeded, the total uptake of labeled palmitate increased. This was associated with a progressive incorporation of radioactivity into platelet phospholipids and glycerides and the appearance of increasing amounts of radioactivity in CO_2. In contrast, the radioactive FFA content of the platelet decreased slightly during the course of the incubation. After 60

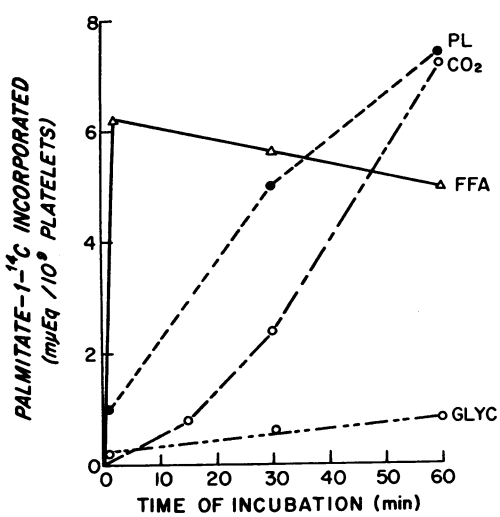

FIGURE 1 Utilization of palmitate-1-^{14} by human platelets. Each point is the mean of two determinations. Incubations were at 37°C with air as the gas phase. The molar ratio of the palmitate-albumin solution was 3.5. Abbreviations are: PL, phospholipids; CO_2, carbon dioxide; FFA, free fatty acid; and G, glycerides.

min, 35% of the *total* fatty acids taken up were oxidized to CO_2, 36% were incorporated into phospholipids, 4% were incorporated into glycerides, and only 25% were present as platelet FFA. Some radioactivity was incorporated into cholesterol esters, but this represented less than 1% of the total uptake and is not shown on the figure. In general, results with oleic acid-1-^{14}C were qualitatively similar to those obtained with palmitate.

Demonstration of net FFA uptake. The uptake of FFA radioactivity was compared with the net change in medium FFA content in order to determine whether the rapid incorporation into the platelet FFA fraction represented a net uptake of fatty acid or resulted from isotopic exchange. Platelets were incubated with palmitate-1-^{14}C–albumin for 2 min, and the total FFA radioactivity and FFA content of the medium before and after incubation was measured (Table I). Incubation with platelets resulted in a 33% decrease in the medium FFA radioactivity and a 28% decrease in the medium FFA content. Only a small change in the medium FFA specific radioactivity was observed, and this difference was not statistically significant. These data indicate that the initial transfer of labeled FFA from albumin to the platelet represents a net uptake of FFA by the cells.

These experiments were designed so that a considerable fraction of the medium FFA content would be taken up by the platelets during a 2 min incubation period. This was necessary in order to produce changes in the medium FFA content that were large enough to be measured accurately by chemical means. Most of the other experiments, which involve only measurements of platelet radioactivity, were designed so that only a small fraction of the total medium FFA content (less than 10%) would be taken up during the entire incubation period.

Comparison between palmitate and albumin uptake. Platelets were incubated with palmitate-1-^{14}C complexed to albumin-^{131}I in order to determine whether FFA uptake might result from incorporation of the intact FFA-albumin complex. Before incubation, the ratio of ^{14}C to ^{131}I in the medium was 0.198 ±0.003 (mean ±SE, four determinations). Much more palmitate-1-^{14}C than albumin-^{131}I was taken up by the platelets during 5 min of incubation at 37°C, and the ratio of ^{14}C to ^{131}I in the platelet pellet was 44.7 ±4.5 (mean ±SE, six determinations). Similar results were obtained when platelets were incubated for 5 min with human plasma to which palmitate-1-^{14}C and tracer amounts of albumin-^{131}I had been added. Hence, the bulk of the initial FFA uptake is not secondary to albumin incorporation.

Effect of temperature on FFA uptake. The incorporation of palmitate into the platelet FFA fraction was not altered when the temperature of incubation was lowered from 37° to 0°C. Palmitate-1-^{14}C uptake after 2 min of incubation was 1.96 ±0.04 nEq/10^9 platelets at 0°C and 1.98 ±0.05 nEq/10^9 platelets at 37°C when the palmitate-albumin molar ratio was 2 (mean of four determinations ±SE). The temperature independence of palmitate uptake refers only to uptake in unesterified form. When the incubation was prolonged, a much larger *total* uptake of palmitate occurred at 37°C as compared with 0°C. However, in these cases, the major effect of an elevation in temperature was to greatly accelerate further metabolism of palmitate, i.e., almost no palmitate was oxidized to CO_2 or incorporated into tissue lipid esters at 0°C.

Effect of FFA concentration on uptake. FFA concentration was varied by adding increasing amounts of fatty acid to a fixed amount of albumin. As shown in Table II, changes in the molar ratio of the incubation medium affected palmitate uptake and utilization by

TABLE I

Comparison Between Uptake of FFA and FFA Radioactivity from the Incubation Medium

Parameter	Value*		Significance of difference
	Before incubation	After incubation‡	
Medium FFA radioactivity, *dpm*	43,200 ±170	28,800 ±590	$P < 0.01$
Percentage decrease in FFA radioactivity, %	—	33	—
Medium FFA content, *nEq*	200 ±5.2	144 ±9.1	$P < 0.01$
Percentage decrease in FFA, %	—	28	—
Medium FFA specific radioactivity, *dpm/nEq*	218 ±5.6	202 ±14.5	$P > 0.1$
Percentage decrease in FFA specific radioactivity, %	—	7	—

* Mean of 16 determinations ±SE.

‡ Incubation was for 2 min at 37°C with air as the gas phase. The molar ratio of palmitate to albumin in the medium before incubation was 5.0.

TABLE II

*Effect of FFA-Albumin Molar Ratio on FFA Uptake and Utilization**

Molar ratio	Palmitate-1-^{14}C incorporated by platelets‡			
	CO_2	Glycerides	Phospholipids	FFA
	nEq/10⁹ platelets			
0.7	5.8 ±0.4	0.75 ±0.2	3.8 ±0.3	0.6 ±0.1
1.0	6.5 ±0.6	0.84 ±0.2	4.1 ±0.3	0.9 ±0.2
1.7	7.4 ±0.8	1.1 ±0.3	5.0 ±0.5	1.7 ±0.3§
2.9	8.3 ±0.8§	1.5 ±0.5	6.1 ±1.0§	3.7 ±0.8‖

* Incubation for 45 min at 37°C with air as the gas phase.
‡ Each value is the mean of six determinations ±SE.
§ Significantly different from $\bar{\nu} = 0.7$ value ($P < 0.05$).
‖ Significantly different from $\bar{\nu} = 0.7$ value ($P < 0.01$).

the platelets. When the molar ratio was increased from 0.7 to 2.9, the quantity of palmitate oxidized to CO_2 in 45 min increased by 43% ($P < 0.05$). Likewise, palmitate incorporation into glycerides increased by 100% ($P > 0.1$) and incorporation into phospholipids increased by 61% ($P < 0.05$). In contrast to these relatively small increases, the magnitude of palmitate uptake in unesterified form increased 620% ($P < 0.01$) when the molar ratio was raised from 0.7 to 2.9. In these experiments, the total amount of palmitate incorporated into CO_2, phospholipids, and glycerides far exceeded that present as platelet FFA. This is due to the fact that the incubations were carried out for 45 min, enough time to permit considerable amounts of FFA to be metabolized. Variations in the FFA-albumin molar ratio over this range also produced large differences in the platelet FFA content in short-term incubations where the bulk of the fatty acid uptake is present as platelet FFA. Hence, the major change produced by increases in the extracellular FFA concentration (i.e. FFA-albumin molar ratio) is an increase in the percentage of the FFA uptake that remains in unesterified form. On the other hand, increases in molar ratio produced a much smaller percentage increase in fatty acid oxidation and esterification.

In additional experiments, the amounts of palmitate and albumin added to the medium were raised in parallel so that the molar ratio of palmitate to albumin remained constant. The time of incubation was 45 min. A 5-fold increase in the concentration of the palmitate-albumin complex (from 0.1 to 0.5 mM albumin) produced only an 18% increase in palmitate uptake. Therefore, as with other isolated cell systems (14), FFA uptake in the platelet appears to be regulated primarily by the FFA-albumin molar ratio rather than by the concentration of the FFA-albumin complex.

The above experiments were performed in incubation media in which albumin was the only protein. Since plasma low-density lipoproteins bind FFA when the FFA concentration is elevated (21, 22), it was conceivable that elevations in FFA content of intact plasma might not result in similar marked increases in the platelet FFA content. In order to examine this question, platelets were incubated with human plasma to which increasing amounts of labeled palmitate had been added in vitro (13). Palmitate-1-^{14}C uptake increased by 100- to 120-fold as the palmitate concentration of the plasma was raised from 0.17 to 2.0 μEq/ml. The bulk of the palmitate-1-^{14}C uptake was recovered as platelet FFA. This is in agreement with the fact that the time of incubation was short (5 min) and the plasma FFA concentrations were high. Therefore, increases in extracellular FFA concentration produced similar results when either plasma or an artificial medium containing albumin as the only protein was employed.

Comparative utilization of different FFA. The uptake and utilization of palmitate, stearate, oleate, and linoleate were compared at FFA-albumin molar ratios of 2 and 4 (Table III). With each acid, greater utilization occurred at molar ratio 4 as compared with molar ratio 2. However, in no case was the incorporation into CO_2, triglycerides, or phospholipids more than 60% higher at molar ratio 4 as compared with molar ratio 2. The largest differences noted were between palmitate and linoleate, and even here, only twice as much linoleate was oxidized to CO_2 and incorporated into phospholipids as compared with palmitate. In contrast, striking percentage increases were noted in fatty acid uptake as platelet FFA. For example, when the molar ratio was increased from 2 to 4, the increments in uptake as FFA were: palmitate, 270%; stearate, 590%; oleate, 290%; linoleate, 400%. Moreover, large differences in uptake as FFA of the saturated and unsaturated fatty acids were noted. For example, when the molar ratio was 2, 4 times as much stearate and 10 times as much palmitate as compared with linoleate were taken up as platelet FFA. When the molar ratio was 4, the stearate uptake was 5.7 times greater and the palmitate uptake 6.6 times greater than that of linoleate. Similar differences in the amounts of saturated and unsaturated fatty acid associated with the platelet as FFA also were observed when the incubation was performed at 0°C. Under these conditions, the further metabolism of fatty acid by the platelet is almost completely inhibited. When the FFA-albumin molar ratio was 3, palmitate uptake as FFA was 3.75 ±0.14 nEq, and that of oleate was 1.36 ±0.09 nEq/10^9 platelets (mean of eight determinations ±SE, $P < 0.01$).

The distribution of radioactivity in platelet phospholipids was similar with each of the four fatty acids. From 75 to 86% of the phospholipid radioactivity was recovered in lecithin, 3 to 7% in phosphadtidyl ethanol-

TABLE III

*Comparison of the Uptake and Utilization of Long-Chain FFA**

FFA	No. of determinations‡	Incorporation§ CO_2 $\bar{v}=2$‖	CO_2 $\bar{v}=4$	Glycerides $\bar{v}=2$	Glycerides $\bar{v}=4$	Phospholipids $\bar{v}=2$	Phospholipids $\bar{v}=4$	FFA $\bar{v}=2$	FFA $\bar{v}=4$
		nEq/10⁹ platelets in 30 min							
Palmitate-1-^{14}C	5	2.4 ±0.2	3.4 ±0.3	0.22 ±0.07	0.30 ±0.2	1.6 ±0.2	2.9 ±0.2	2.1 ±0.2	5.6 ±0.4
Stearate-1-^{14}C	4	3.7 ±0.3	3.6 ±0.3	0.19 ±0.03	0.32 ±0.04	2.2 ±0.2	3.1 ±0.3	0.82 ±0.06	4.8 ±0.5
Oleate-1-^{14}C	5	3.7 ±0.1	4.4 ±0.3	0.20 ±0.06	0.28 ±0.04	2.6 ±0.3	3.7 ±0.1	0.48 ±0.08	1.4 ±0.1
Linoleate-1-^{14}C	4	4.8 ±0.5	5.0 ±0.5	0.26 ±0.09	0.34 ±0.01	3.8 ±0.3	6.1 ±0.6	0.21 ±0.06	0.84 ±0.40

* Incubation at 37°C for 30 min with air as the gas phase.

‡ Each determination was made in duplicate with a different platelet suspension. Four of the suspensions were incubated with each of the four fatty acids, but the last suspension was tested only with palmitate and oleate.

§ Mean ±SE. Only very small amounts of FFA were incorporated into cholesterol esters, and these data have been omitted from this table.

‖ FFA-albumin molar ratio before incubation.

amine, 1 to 8% in sphingomyelin, and 5 to 14% in other phospholipids. These data were obtained from incubations in which the FFA-albumin molar ratio was 4, but similar results also were observed when other molar ratios were tested. For example, at molar ratio 2.8, 88% of the labeled palmitate incorporated into phospholipids was recovered in lecithin. Likewise, at molar ratio 2, 84% of the labeled oleate and 83% of the labeled linoleate incorporated into platelet phospholipids were found in lecithin.

Release of newly incorporated FFA. When platelets were "loaded" with labeled FFA, much of the newly incorporated radioactivity was available for release to a medium containing albumin. Essentially all of the radioactive release occurred within the first 0.5 min of incubation. This was true when the incubation was done at either 0° or 37°C. Furthermore, the quantity of radioactivity released to the medium was almost identical when the platelets were incubated at 0°C as compared with 37°C. In platelets that were loaded with palmitate-1-^{14}C, from 94 to 98% of the released radioactivity was recovered as FFA. Likewise, with oleate-1-^{14}C, 98% of the radioactivity that was released was recovered as FFA. The amount of radioactivity that was released was almost identical with that present as platelet FFA before incubation in albumin. This was true when the platelets were "loaded" with a palmitate-1-^{14}C–albumin solution of either low or high molar ratio. Only the platelet FFA fraction contained sufficient radioactivity to be the primary source of the radioactive FFA that was released. The simplest explanation consistent with these findings is that (*a*) the released FFA is derived from platelet FFA, and (*b*) essentially all of the fatty acid associated with the platelet as FFA is available for very rapid release.

DISCUSSION

The major effect of an elevation in extracellular FFA concentration was to increase the amount of fatty acid associated with the platelets as FFA. Although fatty acid oxidation to CO_2 and esterification into platelet lipid esters also increased as the extracellular concentration was raised, the percentage increase in fatty acid utilization was considerably less than the percentage increase in uptake as FFA. The rate of FFA uptake far exceeded that of fatty acid oxidation and esterification. Therefore, the concentration of FFA in the surrounding medium rather than the rate of fatty acid metabolism by the platelet appears to be the most important factor that regulates the platelet FFA content. As indicated by the experiments in which both palmitate and albumin were labeled, most of the FFA incorporated by the platelets dissociated from albumin in the process of uptake. Like the human erythrocyte (4, 23, 24), the platelet appears to have a much lesser affinity than the primary binding sites of human albumin for FFA (25). This is indicated by the fact that relatively little of the available FFA is associated with the platelets when the FFA-albumin molar ratio is low. Under these conditions, oxidation and esterification occur rapidly enough to remove the small amounts of FFA that are available to the platelets, and the steady-state FFA content of the platelet is very low. As the plasma FFA concentration increases, more of the fatty acid is bound at the weaker binding sites of albumin (11, 23), and cells can begin to compete effectively for this additional FFA (25). Hence, a much larger fraction of the FFA is available for uptake by the platelets. Although exogenous FFA oxidation and esterification also increase when more FFA is available, these increases are not commensurate with the greater FFA uptake, and FFA accumulates in the platelet. Similar results have been noted with other tissues (4, 5).

At a given concentration, considerably more of the saturated fatty acids, palmitate and stearate, were associated with the platelets as FFA as compared with the unsaturated acids, oleate and linoleate. Larger uptake of the saturated FFA also has been observed in Ehrlich ascites tumor cells (5, 26) and human erythrocytes (4). These differences in platelet FFA content cannot be explained entirely by differences in the affinity of albumin for the acids (11, 24). For example, human serum albumin binds oleate much more firmly than linoleate (24). Yet, more oleate than linoleate accumulated in unesterified form in the platelet. Likewise, albumin binds palmitate more tightly than stearate, but more palmitate than stearate accumulated as platelet FFA. These observations suggest that, like albumin, platelets also have a somewhat different affinity for each of the fatty acids.

Studies with the human erythrocyte indicate that the fatty acid taken up as FFA is located on or in the cell membrane (23). The erythrocyte FFA appears to be present in two pools, one at the surface of the plasma membrane and another at an internal location, probably within the membrane (24). Studies with Ehrlich ascites tumor cells (14, 26), acanthamoebae (27), and rat diaphragm (28) are compatible with this interpretation. When these tissues were exposed to an albumin solution, only a portion of the newly incorporated FFA was released into the medium. Only this "exchangeable" or "reversibly bound" fraction of the tissue FFA is considered to be located at the cell surface (24, 26). There is evidence to suggest that passage of FFA into the nonexchangeable pool requires the production of metabolic energy (24, 29–31). In contrast to the results with other tissues, almost all of the newly incorporated platelet FFA was released rapidly when the cells were exposed to albumin. Furthermore, a decrease in the temperature of incubation from 37° to 0°C did not significantly alter FFA uptake in platelets. These findings suggest that almost all of the platelet FFA content is located at or near the cell surface. Therefore, fatty acid uptake as FFA may represent a physical adsorption or binding of the FFA to the platelet membrane.

Most of the FFA that was incorporated into platelet lipid esters was recovered in lecithin. These results agree with those of Andreoli (32, 33), Cohen (8), and Deykin and Desser (7). The latter authors have expressed palmitate incorporation into lecithin as a fraction of total palmitate incorporated into platelet lipids. They found that 54% of the palmitate uptake was recovered in lecithin (7). Recalculation of their data to express the amount in lecithin relative to that in phospholipids rather than in total platelet lipids indicates that 72% of the labeled palmitate incorporated into phospholipids was present in lecithin. This is in good agreement with the value of 75–86% that we obtained. The ratio of FFA incorporation into lecithin as compared with phosphatidyl ethanolamine varied from 13 to 29 for the four long-chain FFA that we tested. In contrast, lecithin–phosphatidyl ethanolamine incorporation ratios ranging from 2.0 to 6.5 were obtained by Cohen (8). These differences may be explained in part by the fact that Cohen incubated platelets with homogenous dispersions of FFA prepared by ultrasonication (8) whereas we used FFA-albumin solutions.

A possible relationship between the platelet FFA content and platelet aggregation is suggested by the present results. It was necessary to prevent platelet aggregation in the present isotopic experiments, for this would have led to trapping of radioactivity in the platelet pellet and, therefore, erroneous results. Aggregation was prevented through the use of a calcium-free artificial medium or plasma containing EDTA (10). Previous studies with Ehrlich cells have demonstrated that removal of calcium from the incubation medium does not appreciably alter FFA uptake (14). The variations in the amount of each acid taken up as FFA correlate well with the ability of the acids to produce aggregation. For example, at a given concentration, much more of the saturated fatty acids, palmitate and stearate, associated with the platelets as FFA. These acids in high concentrations are considerably more potent than the unsaturated acids in inducing aggregation (9, 10). Moreover, as the FFA-albumin molar ratio was raised, the percentage increase in uptake as platelet FFA increased markedly whereas the percentage increases in FFA oxidation and esterification were much less pronounced. The capacity of FFA to produce aggregation also increases markedly as the FFA-albumin molar ratio is raised (10). Taken together, these findings suggest that the ability of high concentrations of saturated FFA to predispose to platelet aggregation may be related to their relatively large uptake as FFA.

ACKNOWLEDGMENTS

These studies were supported by grants from the National Institutes of Health (HE 11,485 and MO1-FR-59) and the Iowa Heart Association. Doctors Spector and Hoak are recipients of Research Career Development Awards K4-HE-20,338 and K3-HE-19,370, respectively, from the National Heart Institute.

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