Method for continuous intravenous infusion of large amounts of oleic acid into rats

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ABSTRACT A method has been developed for the continuous intravenous infusion of large amounts of oleic acid into rats. The acid was infused in the form of an emulsion prepared by sonication and stabilized with albumin in low concentration. Fatty acid was infused at a rate equal to the turnover rate of endogenous free fatty acids and the infusion was continued for 3 hr. During this time there was no evidence of hemolysis or hemoglobinuria; only on occasion did a small clot form at the tip of the infusion catheter.

The infused fatty acids became attached to the circulating albumin and were removed from the plasma and metabolized in the same way as endogenous free fatty acids. There was no evidence to indicate that the infused fatty acids lodged as emboli in the microcirculation or were phagocytized by the reticuloendothelial system.

This method makes it possible to study the direct effects of an increased flux of free fatty acids upon such processes as formation and release of triglyceride by the liver, gluconeogenesis, lipogenesis, ketone body production, glucose utilization, and insulin production.

SUPPLEMENTARY KEY WORDS fatty acids · emulsion · free fatty acids · turnover rate

UNTIL NOW, there has been no simple method for determining in vivo the direct effects of an increased turnover rate of free fatty acids (FFA) on various aspects of lipid and carbohydrate metabolism. FFA in the circulation may be derived from the animal itself (endogenous), or from an intravenous infusion (exogenous). It has been possible to increase the turnover rate of FFA endogenously by altering the nutritional state or by giving the subject various hormones and pharmacologic agents (1–5). Increases achieved by this approach, however, are accompanied by other metabolic changes which prevent a study of the direct effects of the increased turnover rate of FFA. The purpose of this investigation, therefore, was to develop a simple technique which would permit the study of the direct effects of an increased flux of FFA.

As there are no known means of avoiding the secondary changes that develop when the turnover rate of FFA is increased endogenously, the research to be described necessarily began with the premise that the use of an exogenous source is essential if the direct effects of an increased flux of FFA are to be studied. Until recently, all attempts to provide a high concentration of fatty acids (FA) from an exogenous source have failed. Investigators have recognized that if the FA were infused bound to albumin in the same molecular ratio existing in the circulation, prohibitive quantities of albumin would be required. The attempts were limited, therefore, to presenting high concentrations of FA to the circulation as soap. In all of these studies, the FA were toxic to the animal. Death usually occurred very rapidly and was associated with hemolysis and intravenous clot formation (6, 7).

In 1967, Greenough, Crispin, and Steinberg (8) developed the first method for continuously infusing large amounts of FA into the animal nontoxically. Their technique, however, has disadvantages. The method is complex, in that it requires the use of a specially constructed continuous-flow centrifuge. In addition it requires heparin which, by activating lipoprotein lipase—which catalyzes the hydrolysis of triglyceride FA to glycerol and FA—affects lipid metabolism.

For the study of direct metabolic effects of an increased flux of plasma FFA it was necessary to develop an exogenous preparation of FA suitable for infusion. The de-

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Abbreviations: FA, fatty acid(s); FFA, free fatty acid(s).

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sirable conditions for such an infusion are as follows. (*a*) Large amounts of FA must be infused without using large quantities of albumin. (*b*) The infused FA must be metabolized in a similar manner to the endogenous FFA. (*c*) No pharmacologic agents should be associated with the infusion. (*d*) If the infusion of large amounts of FA is to result in an increase in the flux of FFA it must not be accompanied by a compensating decrease in the flux of *endogenous* FFA.

In seeking a method that would fulfill these conditions, I drew on the following observations from previous work. (*a*) FA are transported to the circulation as an FA–albumin complex having a molecular ratio no greater than 7:1 (9). (*b*) The process by which FA combines with albumin is very rapid and does not require energy (9, 10). (*c*) The fractional turnover rate of circulating FFA is high (11–13).

In view of (*b*) and (*c*), I theorized that I could infuse FA and albumin in a molecular ratio much greater than 7:1 if the FA, rather than being complexed to albumin, were prepared as an emulsion, *stabilized* with albumin; for the high turnover rate of circulating FFA and the rapid process of combination of FA with albumin would mean that sufficient circulating albumin would always be available to bind the incoming microspheres of FA immediately.

The method to be described is a successful test of this theory. Amounts of exogenous FA equal to the turnover rate of endogenous FFA were infused without large quantities of albumin. The infused FA quickly formed a complex with the circulating albumin and were not toxic. The method was simple and quite reproducible. It permitted the continuous intravenous infusion of enough FA to increase the turnover rate of plasma FFA by 60%. This increase was achieved by supplying FA from an exogenous source, and without the use of pharmacologic agents having secondary metabolic effects.

METHODS

Preparation of the Fatty Acid Emulsion

The emulsion was prepared as follows: 3.6 ml of 0.15 M sodium chloride, 7.2 ml of distilled water, and 3.6 ml of crystalline bovine albumin (200 mg/ml; pH 5.2) dissolved in 0.15 M sodium chloride were placed in a 25 ml heavy-walled homogenizer tube. A total of 5.4 mmoles of oleic acid (Applied Science Laboratories Inc., State College, Pa.) and a trace amount of oleic acid-1-^{14}C (New England Nuclear Corp., Boston, Mass.) were then added to the mixture in three equal parts. After each of these three additions, the mixture went through the following process of sonication and cooling. A Branson probe sonifier (model S75, Branson Instruments, Inc. Stamford, Conn.) at a power setting of 8 was turned on and tuned in. The probe of the sonifier was then passed through the surface of the liquid mixture, dispersing the FA floating at the top. When the probe was in the mixture and its tip well below the surface, the sonifier was again tuned in and the mixture subjected to sonication for 12–15 sec. When this period of sonication was completed, two precautionary measures were necessary. First, the sonifier was turned off prior to removing the probe from the emulsion. Second, the emulsion was placed in crushed ice for a period of no more than 5 sec. Prolonged cooling resulted in the coalescence of the FA globules in the emulsion. These measures, which inhibited the aeration of the emulsion and dissipated the generated heat, were taken to prevent the peroxidation of the oleic acid, which could cause hemolysis of red blood cells (14). The success of our precautionary measures was indicated by the fact that the FA emulsions used in this study were found to be quite free of peroxides, as determined by iodometric titration with thiosulfate (15) and by thin-layer chromatography (16).

When all of the FA had been added and the mixture sonicated and cooled for the third time, 3.6 ml of 0.45 M sodium chloride was added and the emulsion was sonicated and cooled once again. The FA emulsion was protected from light, kept at room temperature, and infused within an hour after preparation. The final albumin concentration was 40 mg/ml.

In the control studies the infused material was prepared as follows: 3.6 ml of 0.15 M sodium chloride, 7.2 ml of distilled water, 3.6 ml of crystalline bovine albumin (200 mg/ml) to which a trace amount of oleic acid-1-^{14}C had been complexed (17), and 3.6 ml of 0.45 M sodium chloride were mixed together. Thus, the mixture infused in the control experiments was prepared in the same way as the emulsion with three exceptions: the FA (a trace amount of oleic acid-1-^{14}C) were complexed to the albumin, no unlabeled FA were used, and the material did not undergo sonication.

Physical Characteristics of the FA Emulsion

The emulsion, which was a white liquid, had a pH of 4.6 and was stable for about 3 days. The stability of the emulsion was determined by measurement of the concentration of FA at the bottom of the tube containing the emulsion. The concentration remained the same for 3 days and then began to decrease. I assumed that this decrease indicated that the globules of FA in the emulsion were coalescing and rising to the top of the tube.

The size of the FA globules was determined by means of a light microscope. Because the globules coalesce very rapidly under the conditions necessary for viewing them, it was important to place the emulsion on the slide, cover it, and examine it immediately. The globules were generally uniform in size, measuring between 1.2 and

1.8 μ. There were, however, many which measured between 0.2 and 0.4 μ and a few as much as 3 μ. The size depended somewhat on the mode of preparation of the emulsion. When the power setting of the sonifier was decreased from 8 to 6, for example, larger globules were formed.

Intravenous Infusion of the FA Emulsion

The FA emulsion was infused into fed, male Sprague–Dawley rats weighing between 450 and 500 g. The rats were anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.) given by an intraperitoneal injection (50 mg/kg). All the experiments were performed between 10 a.m. and 3 p.m. The emulsion was infused with a Harvard infusion pump (model 600-910/920, Harvard Apparatus Co., Inc., Dover, Mass.) at a rate between 0.021 and 0.033 ml per minute.

After each rat was anesthetized, a tracheotomy was performed and a Teflon catheter (UTX 022 Becton-Dickinson & Co., Rutherford, N. J.) was placed in the left carotid artery. Another catheter made from Silastic tubing (0.020 inch I.D., 0.037 inch O.D., Dow Corning Corp., Midland, Michigan) was inserted into the right femoral vein and threaded into the inferior vena cava so that its tip was about 6 mm above the entrance of the right renal vein.

As unsaturated FA are known to undergo peroxidation when in contact with metal, the infusion system was designed to be free of any metal connections. The distal end of the venous catheter was attached to a length of polyethylene tubing (Clay-Adams, PE 90). This tubing was then connected to a shorter piece of polyethylene tubing (Clay-Adams, PE 300) which was in turn attached to a three-way plastic stopcock connected to a glass-tipped syringe containing the FA emulsion.

One of the major complications I encountered, when attempting to administer a continuous infusion of large amounts of FA, was the formation of a clot adjacent to the tip of the infusing catheter. This complication, I recognized, would develop more readily if there was a back flow and (or) a pooling of blood in the proximal end of the venous catheter. To avoid this, I maintained a constant flow of saline while inserting the catheter into the femoral vein and placing it in the inferior vena cava. The constant flow was maintained by means of a double-barreled infusion pump. One syringe filled with saline was connected to a three-way stopcock, which in turn was connected by a piece of polyethylene tubing to another syringe containing the FA emulsion. The infusion pump was then turned on, saline was carried through the system, and insertion of the venous catheter was begun. When the catheter had been correctly placed in the inferior vena cava, the direction of the three-way stopcock was changed so that the FA emulsion began to flow through the system into the inferior vena cava.

With practice it was possible to complete the insertion and location of the venous catheter in about 5 min. During this time no more than 0.2 ml of saline was infused into the rat.

Periodically, during the infusion, blood samples were collected from the carotid artery and placed in chilled tubes containing a small amount of heparin. No more than 7.5 ml of blood was drawn over a 3 hr period. Samples of tissue taken at the end of the experiment were removed, weighed, and homogenized. All tissues were extracted in chloroform–methanol 2:1. ^{131}I-labeled albumin (Abbott Laboratories) was used to determine plasma volume. Data obtained from the use of ^{131}I permitted appropriate correction for the contamination of tissue by the labeled and unlabeled FFA that was present in the plasma.

Analytical Methods

In all experiments the animals were examined for possible development of complications caused by the infusion of the FA emulsion. The gross appearance of the lung, liver, kidney, spleen, plasma, and urine was noted. Before the final technique was devised, three major complications arose whenever the FA emulsion was infused: hemolysis, hemoglobinuria, and the formation of a clot adjacent to the tip of the catheter. Their seriousness was roughly assessed by weighing the clot and grading the hemolysis and hemoglobinuria on a scale from 0 to 4 plus.

Blood samples were centrifuged promptly and plasma was processed within a few hours of collection. The plasma lipoproteins were separated by ultracentrifugation according to the method of Havel, Eder, and Bragdon (18). The lipid classes were separated by silicic acid column chromatography (19). The FFA were titrated according to the method of Dole (20) as modified by Salamon and Robinson (21). The glyceride-glycerol was determined by the Carlson method (22). Lipid phosphorus was measured by the method of Stewart and Hendry (23). The composition of plasma FFA was determined after their conversion to methyl esters (19) by gas–liquid chromatography on a 12 ft column of ethylene glycol succinate polyester on Chromosorb W (AW) (Applied Science Laboratories) at 186°C, with nitrogen as the carrier gas. The effluent passed through a flame ionization detector, which gave a linear response with mass, and the area of the peaks was simultaneously calculated by an electronic integrator. The values were corrected for variable sensitivity of the detector to different methyl esters on the basis of analyses of known mixtures of methyl esters obtained from Applied Science Laboratories. Information from the gas–liquid chromatograph

was put on punch cards and processed on an IBM 360 model 50 computer by means of a Fortran program (J. Goerke and L. Schmidt, unpublished work).

^{14}C content of the lipids was determined in a Packard liquid scintillation spectrophotometer; ^{131}I activity was determined in a crystal scintillation well counter.

Calculations

FFA turnover rate was calculated as follows (24). The fractional turnover rate was determined by dividing the rate of infusion of oleic acid-1-^{14}C (cpm/min) by the total radioactivity in plasma FFA (cpm/ml × plasma volume in milliliters). The rate of influx of FFA into plasma (μmoles/min) was determined by dividing the rate of infusion of oleic acid-1-^{14}C by the specific activity of plasma FFA. In the steady state the rate of influx of FFA would be equal to the rate of efflux and, therefore, the rate of influx would represent the turnover rate.

RESULTS

The success of an intravenous infusion of high concentration of FA depends upon the prevention of three major complications—hemolysis, hemoglobinuria, and clot formation—which can be avoided by the correct choice for the following four variables: location of the venous catheter, concentration of the infused albumin, composition of the infused FA, and concentration of the infused FA. Hemolysis, hemoglobinura, and clot formation were greatly diminished if the venous catheter was placed between 5 and 7 mm above the right renal vein, if the emulsion contained albumin in a final concentration of 40 mg/ml, and if the emulsion contained oleic acid in a final concentration of 285–300 μmoles/ml. This combination (Table 1) was used as the standard technique, which served as a basis of comparison with other combinations. By varying one of the four factors at a time, while the others were held constant, and then comparing the results with those of the standard system (Tables 2, 3), it was possible to develop a combination in which virtually all complications had been eliminated.

The data in Table 3 demonstrated that the best system for continuous infusion of high concentration of FA was that containing oleic acid in a concentration of 300 μmoles/ml. The pattern observed in the rats receiving the FA emulsion at a rate of 7.6 μmoles/min differed from that observed in the groups receiving 4.8 and 6.3 μmoles/min in that two of the five animals suddenly died 2 hr after the infusion was begun. There was no hemolysis in the blood drawn about 20 min prior to death. Gross examination of the animals did not reveal the cause of death.

To determine whether the virtual absence of gross complications indicated that the infused FA were being metabolized in a physiologic manner, I carried out biochemical studies (Tables 4–9). A comparison was made of the effects of labeled oleic acid infused in trace amounts (control group) with oleic acid infused in high concentration (300 μmoles/ml) in the form of an emulsion (experimental group). As seen in Table 4, the concentration of plasma FFA in the rats receiving the FA emulsion increased during the course of the infusion and was proportional to the amount of FA infused. The plasma FFA rose slightly in control rate during the 1st hr of infusion.

To determine if the infused FA quickly formed a complex with the circulating albumin and was subsequently metabolized by the rat, I determined the distribution of FA between protein, lipoprotein, and nonprotein portions of the plasma (Table 5). In the emulsion, most of the fatty acid was in the $d < 1.006$ fraction and less than 3% was associated with albumin ($d>1.006$). By contrast, 10 min after infusion of the FA emulsion, less than 3% of the plasma fatty acid was truly "free," 75–85% seemed to be associated with albumin ($d>1.21$), and 10–20% with lipoproteins ($d<1.21$; amount in the $d<1.006$ fraction subtracted). In the control rats, into which only a trace amount of labeled oleic acid was injected, the distribution both of mass and radioactivity was similar.

Since the FA was infused as an emulsion composed of FA globules, a major portion of these globules might have lodged in the microcirculation of various organs and (or) been removed by the reticuloendothelial system. However (Table 6), the concentrations of FA in the lungs, kidney, liver, spleen, and adipose tissue were similar in control and experimental rats, as were the percentages of radioactivity taken up by each tissue (Table 7). The highest percentage determined was in liver triglycerides, both in control and experimental animals. Lung, kidney, and spleen lipids contained less than 3% of the total administered after 3 hr of infusion.

TABLE 1 PREPARATION AND INFUSION OF OLEIC ACID EMULSION

	Amounts	Final Concentration
Composition		
Sodium chloride 0.15 M	3.6 ml	—
Distilled water	7.2 ml	—
Crystalline bovine albumin (200 mg/ml) in 0.15 M sodium chloride	3.6 ml	40 mg/ml
Oleic acid	5.4 μmoles	300 μeq/ml
Sodium chloride 0.45 M	3.6 ml	—
Force of sonication	8	
Rate of infusion	0.021 ml/min	—
Location of catheter tip	5–7 mm above right renal vein	—

TABLE 2 EFFECTS OF THREE VARIABLES UPON THE APPEARANCE OF MAJOR COMPLICATIONS*

	Number of Rats	Clot Formed	Hemolysis	Hemoglobinuria
		mg (mean values and range)		
Location of catheter tip				
Above renal veins	6	74 (25–140)	0–tr.	0–1+
Below renal veins	7	146 (100–280)	3+–4+	3+–4+
Albumin concentration (mg/ml) in infusate				
10	5	210 (190–240)	2+–3+	4+
20	4	116 (60–160)	0–tr.	0–1+
30	4	114 (45–160)	0–tr.	0–tr.
40	6	74 (25–140)	0–tr.	0–1+
50	3	120 (110–130)	tr.–3+	4+
Fatty acid composition (%)				
Oleic Linoleic Octanoic				
100 — —	6	74 (25–140)	0–tr.	0–1+
66 34 —	6	153 (100–250)	2+–3+	3+–4+
75 — 25	4	160 (100–195)	tr.–2+	2+–4+
90 — 10	3	215 (180–250)	2+–3+	3+–4+
95 — 5	3	298 (245–350)	2+–3+	3+–4+

* FA emulsion as in Table 1, but containing 230 μmoles/ml, was infused at 4.8 μmoles/min for 3 hr.

TABLE 3 EFFECTS OF OLEIC ACID CONCENTRATION AND RATE OF INFUSION OF THE EMULSION UPON THE APPEARANCE OF MAJOR COMPLICATIONS*

Oleic Acid Concentration	Oleic Acid Infused	Rate of Infusion	Number of Rats	Clot Formed	Hemolysis	Hemoglobinuria
μmoles/ml	*μmoles/min*	*ml/min*		*mg (mean values and range)*		
230	4.8	0.021	6	74 (25–140)	0–tr.	0–1+
300	6.3	0.021	7	19 (0–65)	0–tr.	0
230	7.6	0.033	5	22 (0–55)	0	0

* The oleic acid emulsion was infused for 3 hr.

To determine if FA continued to be released from endogenous sources during the infusion of the FA emulsion, I obtained the following data. The composition of plasma FFA was analyzed by gas–liquid chromatography. The data presented in Table 8 indicate that during the infusion of the FA emulsion the percentage of palmitic acid decreased by approximately 35% while the percentage of oleic acid increased somewhat over 100%. The specific activity of the FA in the emulsion was compared to the specific activity of plasma FFA. The results presented in Table 9 demonstrate that the specific activity of the plasma FFA was consistently lower than that of the FA in the emulsion.

Data and calculations pertaining to the turnover of FFA are summarized in Table 9. The turnover rate of FFA was calculated as the average of values determined at two intervals: 10 min and 1 hr; 1 hr and 2 hr; 2 and 3 hr. The difference in the turnover rate of FFA between the experimental and control groups is readily apparent. During the infusion of the FA emulsion, the turnover rate of FFA was between 8.1 and 8.5 μmoles/min, where as the turnover rate of FFA in the control group was between 5.0 and 5.4 μmoles/min.

DISCUSSION

The desiderata given in the Introduction for a method of infusing fatty acids will be considered in turn.

TABLE 4 EFFECT OF AMOUNT OF OLEIC ACID INFUSED ON THE CONCENTRATION OF PLASMA FREE FATTY ACID*

Oleic Acid Infused	Number of Rats	Time in Hours			
		0	1	2	3
μmoles/min		*μmoles/ml*			
4.8	5	0.45 ± 0.05†	0.70 ± 0.03	0.90 ± 0.03	1.10 ± 0.09
6.3	6	0.40 ± 0.05	0.86 ± 0.04	0.98 ± 0.03	1.55 ± 0.09
7.6	5	0.44 ± 0.08	0.93 ± 0.08	1.38 ± 0.02	1.71 ± 0.01
Control	6	0.40 ± 0.03	0.56 ± 0.04	0.57 ± 0.02	0.59 ± 0.01

* The FA emulsion was infused for 3 hr.
† Mean values ± SEM.

TABLE 5 DISTRIBUTION OF RADIOACTIVITY AND MASS IN LIPIDS AFTER ULTRACENTRIFUGAL ANALYSIS OF THE FATTY ACID EMULSION AND OF PLASMA FROM ANIMALS INFUSED WITH THE FATTY ACID EMULSION

Duration of Infusion	Plasma Lipoprotein					
	FFA				TGFA	
	d < 1.006	d > 1.006	d < 1.21	d > 1.21	d < 1.006	d > 1.006
	μmoles/ml				*μmoles/ml*	
*Experimental**						
10 min	0.04–0.05	0.28–0.48	0.10–0.15	0.50–0.62	1.86–2.65	0.12–0.20
3 hr	0.08–0.17	1.02–1.87			2.54–3.50	0.38–0.44
	% of total FFA radioactivity				*% of total TGFA radioactivity*	
10 min	2.1–2.8	96.9–97.2	13.1–22.5	75.8–85.6	—†	
3 hr	1.2–2.1	97.1–98.5			91.9–100	0–9.1
	μmoles/ml				*μmoles/ml*	
Control						
10 min	0.05–0.06	0.30–0.55	0.08–0.12	0.55–0.63	2.18–3.69	0.27–0.38
3 hr	0.02–0.07	0.60–0.70			1.84–3.80	0.18–0.23
	% of total FFA radioactivity				*% of total TGFA radioactivity*	
10 min	1.5–2.1	97.9–98.5	14.3–19.8	77.6–84.2	—†	
3 hr	1.7–2.3	97.7–98.3			91.3–98.5	1.5–8.7

Values represent the range obtained from two rats in the 10-min experiments and four rats in the 3-hr experiments. FFA, free fatty acid; TGFA, triglyceride fatty acid.
* The experimental animals were infused with an emulsion of oleic acid at the rate of 6 μmoles/min. The emulsion was found to contain 283.0 μmoles/ml (97.3% of total radioactivity) in the d < 1.006 fraction and 2.1 μmoles/ml (1.3% of total radioactivity) in the d > 1.006 fraction.
† No measurable radioactivity was present in these fractions 10 min after the constant infusion was started.

Large Amounts of FA Must Be Infused Without Using Large Quantities of Albumin

"Large amounts" were taken to mean an amount per unit of time equal to or greater than the turnover rate of endogenous FFA, and therefore sufficient to at least double the turnover rate. The endogenous turnover rate of plasma FFA in the control animals was calculated to be between 5.0 and 5.4 μmoles/min; in the "final technique," 6.0–6.3 μmoles of FA were infused per minute.

The FA was infused as an emulsion stabilized with albumin rather than as a fatty acid–albumin complex; the molecular ratio of the FA to albumin was 500:1. Under these conditions only 150 mg of albumin were infused over 3 hr, as opposed to the 10.95g that would have been required if the 1134 μmoles of FA had been bound to albumin prior to infusion.

The Infused FA Must Be Metabolized in the Same Way as Endogenous FA

The criteria here were (*a*) that no untoward reactions should occur as a result of the infusion, (*b*) that the infused FA should quickly form a complex with the circulating albumin, and (*c*) that no major portion of the infused FA globules making up the emulsion should lodge as emboli in the microcirculation and (or) be taken up by the reticuloendothelial system.

The infusion of the FA emulsion resulted in no hemolysis or hemoglobinuria. No clot was formed in three of the seven animals studied. In each of the other four, a very small clot weighing between 10 and 65 mg was formed. Finally, the lungs, liver, kidney, and spleen appeared normal.

Since FFA are transported in the circulation bound to

albumin, it seems reasonable to conclude that if the infused FA are to be metabolized normally they must first bind to the circulating albumin.

Ultracentrifugation of the plasma 10 min after infusion shows (Table 5) that 75–85% of the labeled FA were bound to albumin and a further 12–20% to lipoprotein. That the percentages were no higher can be attributed to the fact that the ionic strength of the plasma was increased when the density was adjusted with potassium bromide prior to ultracentrifugation. Goodman and Shafrir have demonstrated that increasing the ionic strength with salt causes a shift of some of the FA from albumin to lipoprotein (25). From the results presented, it can be concluded that the infused FA did in fact bind to the albumin after entering the circulation.

The question now arises, was the FA metabolized in the same way as the endogenously derived FFA? Past studies to determine the manner in which the endogenous FFA are metabolized have used the intravenous infusion of tracer quantities of labeled FA bound to albumin. 30–50% of such infused FA is taken up by the liver, where the FA are rapidly esterified chiefly to triglycerides which, in part, are released from the liver and carried in plasma d<1.006 lipoprotein (17, 26, 27).

In the present study the metabolic pattern of a trace amount of FA bound to albumin was compared with that of the infused FA emulsion. Tables 6 and 7 show that the FA behaved similarly whether infused in trace amounts or in quantity.

Of course, the similarity observed in the metabolic pattern does not prove that all of the infused FA were metabolized normally. For example, a portion of the infused FA globules might have lodged as emboli in the microcirculation and (or) been taken up by the reticuloendothelial system. Since the amounts of the unesterified FA from the emulsion recovered in the lung, spleen, and liver of the experimental animals were very low and almost identical with those found in the control group, I concluded that little or none of the infused FA had lodged in the microcirculation or had been taken up by the reticuloendothelial system, especially since the lung,

TABLE 6 Concentration of Fatty Acid in Tissues of Experimental and Control Groups

Duration of Infusion	Liver	Lung	Spleen	Kidney	Adipose Tissue
hr			*μmole/g (range)*		
*Experimental**					
0	2.7–2.9				
3	2.3–2.8	1.0–1.4	0.6–0.8	1.2–1.5	1.2–1.6
Control					
0	2.5–2.8				
3	2.3–2.8	0.9–1.4	0.5–0.7	1.0–1.3	1.1–1.5

Four rats were studied in each group.
* Experimental animals were infused with oleic acid at the rate of 6.0 μmoles/min.

TABLE 7 Recovery of Administered Radioactivity from Tissue Lipids 10 min and 3 hr After Continuous Infusion of Oleic Acid Emulsion

Tissue	Oleic Acid Infused	Free Fatty Acids		Triglyceride Fatty Acid		Phospholipid Fatty Acid	
		10 min	3 hr	10 min	3 hr	10 min	3 hr
	μmoles/min			*% of radioactivity administered*			
Liver	6.0	2.3–3.4	0.58 (0.32–0.95)	18–24	22 (17–24)	3.6–5.0	3.90 (3.4–4.9)
	Control	0.16–0.18	0.32 (0.16–0.48)	35–40	28 (20–30)	—	5.29 (3.51–6.32)
Lung	6.0	0.89–1.46	0.38 (0.31–0.45)	0.46–0.49	0.19 (0.14–0.23)	0.29–0.36	0.30 (0.22–0.35)
	Control	—	0.13 (0.10–0.16)	—	0.32 (0.28–0.34)	—	0.76 (0.59–0.86)
Spleen	6.0	0.23–0.28	0.08 (0.03–0.10)	0.07–0.10	0.08 (0.06–0.12)	0.04–0.05	0.06 (0.02–0.08)
	Control	—	0.03 (0.02–0.05)	—	0.12 (0.09–0.16)	—	0.08 (0.07–0.10)
Kidney	6.0	0.33–0.35	0.06 (0.03–0.07)	0.03–0.05	0.47 (0.37–0.54)	0.35–0.45	0.37 (0.32–0.40)
	Control	—	0.14 (0.12–0.16)	—	0.50 (0.46–0.72)	—	1.08 (0.72–1.42)
Adipose tissue*	6.0	0.11–0.18	0.08 (0.06–0.11)	2.58–3.49	2.15 (1.40–2.80)	0.11–0.18	0.05 (0.02–0.11)
	Control	—	0.14 (0.12–0.16)	—	2.98 (1.65–5.32)	—	0.07 (0.05–0.08)

Two rats were studied at 10 min and four at 3 hr. Mean values and (or) ranges given.
* Taken as 7% of body weight.

which contains the first microcirculation encountered by the FA emulsion after its entry into the inferior vena cava, did not have high concentrations of FA.

No Pharmacologic Agents Should Be Associated with the Infusion

Since only FA, albumin, and saline were used in the preparation of the emulsion there were no pharmacologic agents which could cause secondary metabolic changes. On this basis, the method presented should permit the direct study of the consequences of the increased flux of FFA upon various aspects of lipid and carbohydrate metabolism. It is true that barbiturate anesthesia was used in both control and experimental studies. It may be possible to avoid this by modifying the experimental technique.

The Release of Endogenous FFA to the Plasma Should Not Be Decreased

If the infusion of FA were associated with a lowered release of endogenous FFA, the turnover rate of plasma FFA would not necessarily be increased. To determine if the release of endogenous FFA continued during the infusion of the FA emulsion, I compared the specific activity of the FA in the emulsion to the specific activity of the plasma FFA. Since the specific activity of the plasma FFA was consistently lower than that of the emulsion,

TABLE 8 FATTY ACID COMPOSITION OF FREE FATTY ACID IN PLASMA

Fatty Acid*	Control Group		Experimental Group	
	10 min	3 hr	10 min	3 hr
	% by weight			
12:0	0	1.0	0	0
14:0	4.4	6.4	6.6	6.0
14:1	0	0	0	0
16:0	39.0	40.9	26.4	24.2
16:1	6.5	5.5	3.6	3.7
18:0	20.0	15.9	13.1	10.1
18:1	16.2	18.5	38.7	45.2
18:2	12.0	10.5	9.7	8.7
18:3	1.5	1.2	1.5	1.2

Values are the averages from two animals in each group.
* Designated by chain length:No. of double bonds.
† Standard technique used as described in Table 1 except that the concentration of oleic acid was 288 μmoles/ml.

TABLE 9 SUMMARY OF DATA RELATING TO TURNOVER OF FREE FATTY ACID

Influx Rate of Oleic Acid-^{14}C	Plasma Volume	Time	Plasma Free Fatty Acids					
			Concentration		Specific Activity	Turnover Rate*	Fractional Turnover Rate*	
							Endogenous and Exogenous	Exogenous
cpm/min†	*ml*		*cpm/ml*	*μmoles/ml*	*cpm/μmole*	*μmoles/min*	*per min*	
Experimental‡								
89,200 (87,000–90,100)	13	10 min	6,870 (5,780–7,000)	0.61 (0.53–0.70)	11,170 (10,500–13,900)			
						8.1	0.93	0.93
	13	1 hr	7,980 (6,560–9,760)	0.74 (0.57–0.86)	10,785 (8,510–12,200)			
						8.1	0.73	0.73
	14	2 hr	10,545 (9,100–11,680)	0.93 (0.80–1.15)	11,340 (10,100–13,100)			
						8.5	0.57	0.51
	15	3 hr	14,390 (9,200–19,450)	1.49 (1.10–2.05)	9,660 (8,660–12,000)			
Control								
100,000 (99,000–102,000)	13	10 min	11,200 (10,000–12,300)	0.59 (0.40–0.75)	18,950 (15,600–19,200)			
						5.4	0.72	
	13	1 hr	10,100 (8,050–11,250)	0.56 (0.38–0.79)	18,500 (15,730–20,000)			
						5.0	0.66	
	14	2 hr	11,750 (8,150–14,790)	0.58 (0.42–0.71)	21,800 (19,200–22,500)			
						5.4	0.60	
	15	3 hr	9,360 (7,230–12,780)	0.64 (0.41–0.78)	16,380 (14,400–17,500)			

Four rats were studied in each group.
* Calculated as the average of values determined at two intervals: 10 min and 1 hr; 1 hr and 2 hr; and 2 hr and 3 hr.
† Mean values and ranges.
‡ Experimental animals were infused with an emulsion of oleic acid [specific activity 14,900 (range 13,700–15,500) cpm/μmole] at the rate of 6.0 μmoles/min.

the release of unlabeled FFA from endogenous sources must have continued during the infusion (though not necessarily at the same rate as before). Additional data to support this contention were obtained from gas–liquid chromatographic analyses of the composition of plasma FFA during the course of the infusion. As the fractional turnover rate of plasma FFA is very rapid, a marked decrease in the release of endogenous FFA during the infusion would result in a change of plasma FFA composition. Although the percentage of palmitic acid was decreased (Table 8) and the percentage of oleic acid increased after the infusion of the FA emulsion, the normal plasma FFA were not entirely replaced by oleic acid. On the basis of these data, I concluded that FFA continues to be released from endogenous sources during infusion.

Evidence has been presented that the conditions for increasing the flux of plasma FFA and studying the direct metabolic effects of the increase have been successfully met. Now it is necessary to determine, by actual calculation, whether or not the turnover rate of FFA was increased. A comparison of the FFA turnover rates of the control and experimental animals should provide the answer.

In the control experiments (those in which a trace amount of labeled oleic acid was infused), the concentrations of labeled and unlabeled plasma FFA remained virtually constant over the 3 hr experimental period (Table 4). Under these steady state conditions, the turnover rate of plasma FFA was calculated to be 5.0–5.4 μmoles/min.

Calculation of the FFA turnover rate in the experimental animals at first seemed impossible since the concentration of plasma FFA increased continuously during the period of infusion. The following calculations demonstrate, however, that the turnover of plasma FFA remained constant. From the data in Table 9 it can be calculated that 16.1×10^6 cpm (1080 μmoles) of oleic acid were infused during the 3-hr experiments. At the end of the study the plasma compartment contained a total of 0.22×10^4 cpm of ^{14}C (cpm/ml $\times$ plasma volume) and had accumulated 14.5 μmoles of FFA (μmoles/ml $\times$ plasma volume minus zero time μmoles/ml $\times$ plasma volume). Thus less than 2% of the infused FA was present in the plasma at the end of the experiment while over 98% of it had been removed. The same relationship was present between the amount of FA radioactivity and mass infused and that removed from the plasma after 1 and 2 hr of infusion. If the endogenous FFA were being removed similarly, the turnover of plasma FFA would be so nearly constant that for purposes of calculation steady-state kinetics could be assumed. The data presented in Table 9 show that the FA infusion increased the turnover rate of plasma FFA by approximately 60%.

When the amount of FA infused per minute (6.0 μmoles/min) is subtracted from the amount of FFA turned over per minute, we arrive at a turnover rate for *endogenous* FFA of 2.1–2.5 μmoles/min, less than half that in the control experiments. Thus although the infusion increased the over-all turnover rate of FFA, this increase was not proportional to the FFA concentration in the plasma, and the turnover, and hence release, of endogenous FFA decreased. The mechanism of the decrease is unknown.

The fractional turnover rate of the exogenously derived FA was calculated by dividing the rate of infusion of radioactivity by the total radioactivity in plasma FFA (Table 9). These fractional turnover rates (0.93, 0.73, and 0.51 per min for early, middle, and late intervals of the 3 hr experiment) were very similar to the fractional turnover rates calculated for the total pool of plasma FFA (endogenous and exogenous) by dividing the turnover rate of FFA (endogenous and exogenous) by the amount of FFA in the plasma. This strongly suggests that the turnover rates of plasma FFA of endogenous and exogenous origin were the same.

In conclusion, these studies demonstrate that the turnover of plasma FFA was increased by the infusion of large amounts of FA (although the turnover of endogenous FFA was simultaneously reduced) and that the FA infused were removed from the plasma and metabolized in the same way as endogenous FFA. It should now be possible to study, in vivo, the effects of an increased turnover rate of FFA on such processes as the formation and release of triglyceride by the liver, gluconeogeneis, lipogenesis, ketone body production, glucose utilization, and insulin production.

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