# Epinephrine Plasma Thresholds for Lipolytic Effects in Man

# MEASUREMENTS OF FATTY ACID TRANSPORT WITH [1-<sup>13</sup>C]PALMITIC ACID

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ABSTRACT To determine the plasma epinephrine thresholds for its lipolytic effects, 60-min epinephrine infusions at nominal rates of 0.1, 0.5, 1.0, 2.5, and 5.0  $\mu$ g/min were performed in each of four normal young adult men while they also received a simultaneous infusion of [1-13C]palmitic acid to estimate inflow transport of plasma free fatty acids. These 20 infusions resulted in steady-state plasma epinephrine concentrations ranging from 12 to 870 pg/ml. Plasma epinephrine thresholds for changes in blood glucose, lactate, and  $\beta$ -hydroxybutyrate were in the 150–200-pg/ml range reported by us previously (Clutter, W. E., D. M. Bier, S. D. Shah, and P. E. Cryer. 1980. J. Clin. Invest. 66: 94-101.). Increments in plasma glycerol and free fatty acids and in the inflow and outflow transport of palmitate, however, occurred at lower plasma epinephrine thresholds in the range of 75 to 125 pg/ml. Palmitate clearance was unaffected at any steady-state epinephrine level produced. These data indicate that (a) the lipolytic effects of epinephrine occur at plasma levels approximately threefold basal values and (b) lipolysis is more sensitive than glycogenolysis to increments in plasma epinephrine.

### INTRODUCTION

The importance of the sympathetic nervous system and epinephrine in the regulation of fat metabolism has been known for more than 40 yr (1-4). Dole (5), and Gordon and Cherkes (6) first demonstrated that epi-

nephrine injection caused a prompt increase in plasma FFA levels, which Havel and Goldfein (7), using  $[1-^{14}C]$  palmitate, showed to be the result of altered FFA transport. The rise in plasma FFA produced by catecholamine administration was subsequently confirmed in many species including man (7-9), dog (7, 10-13), rat (14, 15), monkey (16), and sheep (17, 18).

Although a pulse intravenous injection of epinephrine as small as 0.05  $\mu$ g/kg (7) and an infusion as little as  $0.25 \,\mu$ g/min (19) can increase plasma FFA concentrations, most investigations of catecholamine-induced lipolysis in vivo have used doses that might be expected to produce plasma catecholamine concentrations higher than those occurring under usual physiological conditions. Furthermore, since these studies were carried out before the availability of sensitive isotope derivative methods (20, 21) for measuring plasma epinephrine and norepinephrine concentrations, the plasma catecholamine levels required to produce lipolytic effects have not been well defined. In a recent study (22), we infused adults with graded epinephrine doses to establish physiologic thresholds for this hormone's metabolic and hemodynamic actions. This study showed that plasma epinephrine thresholds for increments of plasma lactate,  $\beta$ -hydroxybutyrate, and glucose (the latter the combined result of an increased glucose production and an impaired glucose clearance) were in the range of 150 to 200 pg/ml. Plasma glycerol, however, increased with an apparent epinephrine threshold of 75-125 pg/ml suggesting that lipolysis was more sensitive to epinephrine than was glycogenolysis. In the present investigation we have pursued the above observation by directly estimating lipolysis with the nonradioactive tracer

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 TABLE I

 Plasma Glucose, Insulin, and Glucagon Values (Mean±SE) before and during Saline or Epinephrine Infusion

Nominal epinephrine		0*			0.1			0.5	
infusion rate, μg/min	Bţ	1 <sub>30</sub> §	I <sub>60</sub> <sup>#</sup>	В	I <sub>30</sub>	I <sub>60</sub>	В	I <sub>30</sub>	I <sub>60</sub>
Glucose, mg/dl	90±2	86±4	87±3	85±1	89±2	88±2	88±4	90±4	93±4
Insulin, $\mu U/ml$	$8\pm 1$	$9\pm 2$	$9\pm 2$	$8\pm 2$	$12 \pm 1$	9±1	9±1	13±3	13±3
Glucagon, pg/ml	$110 \pm 12$	111±8	$105\pm7$	$112 \pm 2$	$108\pm2$	$103\pm5$	$98\pm2$	$105\pm6$	95±8

\* Saline control infusion.

‡ Average of five basal values for each subject before epinephrine infusion.

§ Infusion values 30 min after the start of the epinephrine infusion at the indicated rate.

<sup>II</sup> Infusion values 60 min after the start of the epinephrine infusion at the indicated rate.

[1-<sup>13</sup>C]palmitic acid in young adults during graded epinephrine infusions. The results confirm that epinephrine will accelerate the inflow of FFA into plasma at concentrations approximately half those required to increase glucose production.

#### **METHODS**

Four normal young adult men, aged 22–26 yr, of normal weight (75.5±3 kg) and height (195±3 cm), each consented to six 120-min infusions of [1-<sup>13</sup>C]palmitic acid. During the 2nd h of each study, the subject also received a simultaneous infusion of epinephrine at one of five nominal rates (0.1, 0.5, 1.0, 2.5, and 5.0  $\mu$ g/min) or a saline control infusion. All infusions were performed after an overnight hospitalization during which time the patient was allowed to drink water but otherwise fasted, including abstinence from alcohol, tobacco, and caffeine. Subjects were supine throughout the course of the infusion studies, which were spaced at intervals of at least 1 wk in a given subject. The epinephrine infusion sequence was varied in a fashion unknown to the individual studied.

Three intravenous catheters were placed into superficial veins in each subject 30 min before the start of the study. Two catheters for infusion of [1-13C]palmitate and epinephrine (or saline), were placed in one arm and a sampling catheter was placed in the contralateral arm. 30 min after catheter placement, a continuous infusion of [1-13C]palmitic acid (90 atom percent 1-13C; Merck Sharp and Dohme of Canada Limited, Quebec, Canada) complexed to essentially fatty acidfree human albumin (Sigma Chemical Co., St. Louis, Mo.) (23) was begun at the rate of 0.033 μmol [1-13C]palmitate per kg·min using a calibrated syringe pump (Harvard Apparatus Co., Inc., S. Natick, Mass.). The fatty acid infusion rate was subsequently verified by measuring the palmitic acid content of the infused solution by gas chromatography as described below. 60 min thereafter, appropriate amounts of (-) epinephrine (adrenaline chloride, Parke, Davis & Company, Detroit, Mich.) were diluted in saline containing ascorbic acid (0.5 mg/ ml) and infused at a constant rate with a second syringe pump. Preliminary studies have shown that such infusate epinephrine concentrations are stable for 120 min at room temperature. Stability was confirmed by measuring infusate epinephrine concentrations before and after each infusion.

Blood samples were drawn prior to the onset of the  $[1^{-13}C]$ palmitic acid infusion (-60 min), and then at 10-min intervals from -30 min to the end of the epinephrine infusion (+60 min). A final sample was taken 30 min after cessation of the epinephrine infusion (+90 min). Aliquots were immediately distributed to iced tubes containing heparin (for analysis of FFA and glucose concentration and  $[1^{-13}C]$ palmitate enrichment); EDTA plus aprotinin (for analysis of insulin and glucagon); iced 3 M perchloric acid (for measurement of  $\beta$ -hydroxybutyrate and glycerol); and heparin plus reduced glutathione (for measurement of plasma catecholamines). All tubes were promptly centrifuged in a refrigerated centrifuge and the supernates separated. The fatty acids were extracted immediately from 1-ml samples of heparinized plasma into 5 ml of Dole's mixture (5) containing heptadecanoic acid (0.059  $\mu$ mol/ml) as internal standard (24) for subsequent measurement of FFA concentration and [1-<sup>13</sup>C]palmitate enrichment. The other supernates were frozen for future analysis.

Plasma glucose, glycerol,  $\beta$ -hydroxybutyrate, insulin, glucagon, and catecholamines were measured as previously described (22). Plasma FFA were quantified by the gas chromatography method of Hagenfeldt (24) after conversion to the methyl esters with 1% sulfuric acid in methanol. Fatty acid separation was achieved using a 2-m × 2-mm column of 10% SP-2330 (Supelco Inc., Bellefonte, Pa.) held isothermal at 160°C for 2 min, then programmed to 240°C at 4°C/min. The content of individual FFA was determined by comparison of peak area to the area of the heptadecanoic acid internal standard. Total plasma FFA concentration was recorded as the arithmetic sum of the contents of the individual fatty acids from 12:0 to 20:4. An aliquot of the same methyl ester fraction was analyzed for plasma [1-13C]palmitic acid enrichment by 70 eV electron impact, selected ion monitoring gas chromatography-mass spectrometry of the molecular ion region  $(m/z \ 270 \text{ and } 271)$  of palmitate methyl ester after resolution by isothermal gas chromatography at 150°C on a 2-m × 2-mm 3% SP-2300 column. The ion monitoring was accomplished with a computer-controlled Finnigan 3300 (Finnigan Corp., Sunnyvale, Calif.) quadrupole gas chromatography-mass spectrometry system capable of measuring ion current ratios with a precision of better than  $\pm 1\%$  (25), a precision in the range we attained measuring [1-13C]palmitate enrichments using a manually controlled ion monitoring system (26). Calibration standard solutions of known [1-13C]palmitic acid enrichment were run before each plasma series.

The inflow transport or rate of appearance  $(R_a)^i$  of palmitate into the sampled plasma compartment and its rate of outflow transport or disappearance  $(R_d)$  were calculated by Steele's equations (27). During the basal, preepinephrine infusion steady-state period, these equations reduce to the conventional steady-state radiotracer expression (28)

$$R_a = R_d = turnover = i/E_p$$
(1),

<sup>&</sup>lt;sup>1</sup>Abbreviations used in this paper:  $E_i$ , isotopic enrichment of infused tracer;  $E_p$ , tracer enrichment at plateau;  $p[E]_{ss}$ , steady-state plasma epinephrine concentration;  $R_a$ , rate of appearance;  $R_d$ , rate of disappearance;  $R_c$ , rate of clearance.

 TABLE I (Continued)

	1.0			2.5			5.0	
В	I <sub>30</sub>	I <sub>60</sub>	В	I <sub>30</sub>	I <sub>60</sub>	В	I <sub>30</sub>	I.60
87±5	93±5	98±6	90±2	115±8	$125 \pm 10$	87±2	140±4	$166 \pm 12$
13±3	$17 \pm 6$	15±3	$11 \pm 2$	$16 \pm 4$	$20\pm 2$	$14 \pm 4$	$18 \pm 7$	$23 \pm 9$
$125\pm16$	$107\pm15$	$103 \pm 6$	113±8	$103 \pm 9$	$101 \pm 9$	$104 \pm 7$	$97\pm3$	$91 \pm 9$

where i is the infusion rate of the tracer and  $E_p$  is the tracer enrichment at plateau. For application to studies using stable isotope tracers which, in themselves, contribute slightly to the mass of the substrate pool (~0.5-2.0% in the present studies), and are labeled to an extent <100% (90% in the current instance), the above equation is modified to the form

$$R_{a} = R_{d} = turnover = i \left[ \frac{E_{i}}{E_{p}} - 1 \right]$$
(2),

where  $E_i$  is the enrichment of the [1-1<sup>3</sup>C]palmitate infused (atom percent excess) and the second term in Eq. 2 removes the contribution of the tracer infusion rate from the palmitate turnover (29).

In the nonsteady-state epinephrine infusion period Steele's equation for  $R_a$ , modified for stable isotope use because of the considerations listed above, becomes

$$\mathbf{R_a} = \left[ \frac{\mathbf{i} \cdot \mathbf{E_i} - \mathbf{Q}(t) \left\lfloor \frac{\mathbf{d}}{\mathbf{dt}} \mathbf{E}(t) \right\rfloor}{\mathbf{E}(t)} \right] - \mathbf{i}$$
(3),

where E(t) and Q(t) are the plasma 1-<sup>13</sup>C enrichment and the palmitate pool size, respectively, at time t. The latter pool size was calculated as the product of the plasma concentration times the plasma volume taken as 5% of the body weight (30). Because of the rapid flux of FFA, the tracer was considered to mix completely in the plasma space during the 10-min interval between samples (31).

The palmitate outflow transport or disappearance rate in the nonsteady state was calculated according to Steele (27) as:

$$\mathbf{R}_{\mathbf{d}} = \mathbf{R}_{\mathbf{a}} - \frac{\mathbf{d}}{\mathbf{dt}} \mathbf{Q}(\mathbf{t}),$$

an expression that does not require modification for stable isotope application since the equation contains no isotopic enrichment terms.

Palmitate clearance ( $R_c$ ) was calculated by dividing the palmitate outflow transport ( $R_d$ ) by the concurrent plasma palmitate concentration (28).

Plasma epinephrine thresholds for its metabolic effects were estimated through the use of semilogarithmic plots of the steady-state plasma epinephrine concentration  $(p[E]_{ss})$ to the measured variable (22). This was done on the premise that extrapolation of the central linear portion of the sigmoidal dose-response curve provides a maximal estimate of the threshold for that variable (22). Since the effects of higher epinephrine infusion rates on lipid transport are temporary, threshold responses were calculated from the correlations between steady-state plasma epinephrine concentration (p[E]ss) and the maximal substrate or kinetic value measured during the course of the epinephrine infusion. For palmitate Ra this maximum occurred between 20 and 30 min, for glycerol and FFA concentration between 20 and 40 min; and for glucose, lactate, and  $\beta$ -hydroxybutyrate at 60 min. Since there was significant intra- and intersubject variability

in basal plasma palmitate concentration and turnover on different study days (see Results) the values during the epinephrine infusion are represented as the percent change from each day's average base-line value. Student's t test for paired comparisons was used to test the significance of substrate and flux changes resulting from the epinephrine infusion.

#### RESULTS

During the saline control study, plasma epinephrine concentration averaged  $26.3\pm4.3$  pg/ml (mean $\pm$ SE). Within 10 min of starting epinephrine infusions at nominal rates of 0.1, 0.5, 1.0, 2.5, and 5.0  $\mu$ g/min all subjects reached new p[E]<sub>ss</sub> levels averaging 72.6 $\pm$ 6.1, 102.3 $\pm$ 5.3, 151.1 $\pm$ 9.0, 337.8 $\pm$ 15.6, and 660.6 $\pm$ 34.0 pg/ml, respectively, concentrations comparable to our prior study with the same infusion rates (22). Plasma norepinephrine averaged 211 $\pm$ 7 pg/ml in the basal state and did not change significantly during epinephrine infusion, a result consistent with our previous investigation (22).

The mean ± SE values of plasma glucose, insulin, and glucagon measured before and 30 and 60 min after the start of epinephrine infusion at the five nominal rates are shown in Table I. During the saline control study, plasma glucose and hormonal levels remained unchanged from the basal, preinfusion values. As noted previously (22, 23), epinephrine at the two highest infusion rates produced a significant (P < 0.05, paired t) rise in plasma glucose by 30 min which was accompanied by a slight, but significant (P < 0.05) increase in plasma insulin at the  $2.5 - \mu g/min$  infusion rate. At the 5.0- $\mu$ g/min rate, however, there was wide individual variability in insulin response. Plasma glucagon was not significantly altered at any dose of epinephrine studied, compatible with our prior results (22).

Table II shows the basal plasma fatty acid concentrations and net inflow transport rates ( $R_a$ ) during the five studies in each individual. The average postabsorptive plasma palmitate and FFA concentrations were 132±7 and 549±13.9  $\mu$ M, respectively, values comparable to previously reported levels (32–37). Palmitate comprised an average of 24.2% of the total circulating FFA, a figure that also compares well with reported values (32, 34, 38). The overall mean palmitate

Basal Pl	asma FF.	A Concen	trations (	Micromola	rs) and I	nflow Ra	tes (Micr	romoles pe	er Kilogra	ım per M	inute) in	the Study	Patients			
		Pati	ent l			Patie	ent 2			Patio	ent 3			Patie	ent 4	
Infusion	[16:0]*	[SFFA]*	R <sub>a</sub> 16:0	R <sub>a</sub> Sffat	[16:0]	[2FFA]	R <sub>a</sub> 16:0	R <sub>a</sub> Sffa	[16:0]	[SFFA]	R <sub>a</sub> 16:0	R <sub>a</sub> SFFA	[16:0]	[SFFA]	R <sub>a</sub> 16:0	R <sub>a</sub> Sffa
Saline	117	459	1.69	6.63	147	595	1.74	7.03	120	431	1.84	6.61	131	568	2.13	9.26
Epinephrine, 0.1 μg/min	130	550	2.29	9.69	194	101	1.86	6.74	I	1	1	I	140	635	1.63	7.38
Epinephrine, 0.5 μg/min	68	285	1.23	5.05	208	835	1.88	7.53	130	559	1.49	6.17	147	661	2.05	9.28
Epinephrine, 1.0 μg/min	95	420	1.97	8.70	110	507	1.60	7.35	129	531	2.98	12.23	131	536	2.41	9.80
Epinephrine, 2.5 μg/min	123	551	2.11	9.41	141	605	2.30	9.85	189	708	2.80	10.51	141	584	2.65	11.08
Epinephrine, 5.0 μg/min	60	248	1.37	5.67	175	704	1.94	7.78	125	495	2.10	8.32	107	467	2.20	9.62
Mean	98.8	418.8	1.78	7.53	162.5	657.8	1.89	7.71	138.6	544.8	2.24	8.77	132.8	575.2	2.18	9.40
(SE)	12.1	52.7	0.17	0.82	14.9	46.5	0.10	0.45	12.7	46.1	0.28	1.15	5.8	28.5	0.14	0.49
* Area of palmi to epinephrine t Calculated by as a whole (31,	tic acid c (saline) i / dividing 32).	or of FFA infusion. f the R <sub>a</sub> 16	12:0 to 2 3:0 by the	0:4 relative fraction of	e to hepta plasma Σ	idecanoic EFFA mae	acid (17 de up by	:0) interna palmitate	al standar taken as	d. Averag a represe	ge value ntative tr	of five san acer for fa	nples take tty acid ti	en prior ransport		

TABLE II

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inflow to plasma was  $2.02 \pm 0.11 \,\mu$ mol/kg per min which, since palmitate is typical of FFA transport on the whole (32), represented an average FFA R<sub>a</sub> of  $8.35 \pm 0.44$  $\mu$ mol/kg per min, both values similar to those found in prior investigations (36, 37, 39–41). As noted by others (39, 41–44), the net inflow transport of fatty acids was directly proportional to the plasma concentration (Fig. 1).

Table III shows the plasma FFA, glycerol, and  $\beta$ hydroxybutyrate levels before and during the epinephrine infusion studies. All basal substrate values remained unchanged by the saline control infusion. By paired t analysis, both plasma palmitate and total FFA concentrations were significantly (P < 0.05) elevated at 30 and 60 min by an epinephrine infusion rate of 0.5  $\mu$ g/min or greater. Plasma glycerol was likewise significantly elevated at infusion rates of 1.0  $\mu$ g/min or more, except for the 60-min value at the highest infusion dose. Plasma  $\beta$ -hydroxybutyrate, on the other hand, was only significantly (P < 0.05) increased by epinephrine infusion at the rates of 2.5 and 5.0  $\mu$ g/min.

Fig. 2 illustrates the changes in plasma palmitate concentration and in palmitate inflow and outflow transport induced by epinephrine infusion. At the lowest epinephrine infusion rate  $(0.1 \,\mu g/\text{min})$  there was a slight, but insignificant, rise in plasma palmitate and in palmitate  $R_a$  and  $R_d$  between 20 and 30 min. By paired t analysis, there was a significant (P < 0.05) increase in palmitate net inflow transport ( $R_a$ ) by 15 min at the next lowest ( $0.5 \,\mu g/\text{min}$ ) epinephrine infusion rate. At the higher epinephrine infusion rates (Fig. 2), plasma palmitate rose significantly (P < 0.01) to peak values at 30 to 40 min after the start of the epi-



FIGURE 1 Relationship between basal palmitate inflow transport and palmitate concentration. The linear function  $y = 4.6 \times 10^{-3} x + 1.39$  fits the data with a correlation coefficient of 0.38 (P = 0.04).



FIGURE 2 Plasma palmitate concentration, net inflow  $(R_a)$ , and net outflow  $(R_d)$  transport (mean  $\pm SE$ ) during saline and epinephrine infusions. Values are reported as percent basal (the 60-min period before start of saline or epinephrine infusion) because of intra- and intersubject variability in the nonstimulated values.

nephrine infusion reflecting, in each instance, the net effect of a preceding significant (P < 0.05) increase in  $R_a$  not completely offset by a lesser increase in  $R_d$ . The effect of epinephrine was transient in each instance and 30 min after cessation of epinephrine, plasma palmitate concentrations averaged  $77.0\pm1.2$ ,  $90.8\pm13.8$ ,  $73.8\pm7.8$ ,  $62.7\pm4.3$ , and  $82.5\pm17.0 \ \mu$ M for the 0.1, 0.5, 1.0, 2.5, and 5.0  $\ \mu$ g/min doses, respectively, values similar to the palmitate concentration of  $94.0\pm6.0$  obtained at the same sampling time following saline infusion.

The data used to estimate the plasma epinephrine thresholds for its lipolytic effects are illustrated in Figs. 3 and 4 where the  $p[E]_{ss}$  for each infusion is plotted against the change in plasma palmitate and glycerol concentrations and the change in transport of palmitate into and out of the plasma compartment, during that infusion. Alterations in these variables represent the difference between the average basal value and the corresponding peak value occurring 20– 30 min after the start of the epinephrine infusion.

Increments in plasma FFA and glycerol (Fig. 3) occurred at plasma epinephrine levels in the 75-125-pg/ml range, consistent with a threshold effect in that range observed for glycerol in our previous study (22). Likewise, a similar threshold was observed for palmitate  $R_a$  and  $R_d$  (Fig. 4). Although there was considerable

TABLE III FFA, Glycerol, and  $\beta$ -Hydroxybutyrate Values (Mean ±SE) before and during Saline or Epinephrine Infusion

Nominal epinephrine		0*			0.1			0.5	
infusion rate, μg/min	Bţ	I30§	I <sub>60</sub> ®	В	I <sub>30</sub>	I <sub>60</sub>	В	I <sub>30</sub>	I <sub>so</sub>
Palmitate,¶									
μM	$129 \pm 7$	$127 \pm 11$	$138 \pm 10$	$155 \pm 20$	$183 \pm 33$	$158 \pm 15$	$138 \pm 29$	$271 \pm 62$	$180 \pm 36$
ΣFFA,¶ μM	$513 \pm 26$	$517 \pm 63$	$566 \pm 60$	$629 \pm 44$	$732 \pm 99$	$650 \pm 49$	$585 \pm 115$	$1142 \pm 262$	$778 \pm 146$
Glycerol, $\mu M$	67±17	$72 \pm 18$	$64 \pm 18$	$52 \pm 14$	68±18	$52 \pm 32$	$56 \pm 16$	$85 \pm 29$	$75 \pm 24$
β-OH-butyrate,									
μM	$126 \pm 23$	118±25	$115 \pm 29$	$146 \pm 28$	187±58	$177 \pm 57$	$133 \pm 32$	253±72	278±84

\* Saline control infusion.

‡ Average of five basal values for each subject before epinephrine infusion.

§ Infusion values 30 min after the start of the epinephrine infusion at the indicated rate.

<sup>II</sup> Infusion values 60 min after the start of the epinephrine infusion at the indicated rate.

¶ Area of palmitic acid or of FFA 12:0 to 20:4 relative to heptadecanoic acid (17:0) internal standard.

individual variation, an increase in palmitate  $R_a$  ( $\geq 0.5 \ \mu mol/kg$  per min) was observed in all subjects with  $p[E]_{ss} > 75 \text{ pg/ml}$ . On the whole, a similar but lesser increase occurred in  $R_d$  but five subjects with  $p[E]_{ss} < 75 \text{ pg/ml}$  showed essentially no change in palmitate outflow transport ( $\Delta R_d \leq 0.5 \ \mu mol/kg$  per min). In all instances except two, however,  $\Delta$  palmitate  $R_a$  exceeded  $\Delta$  palmitate  $R_d$  and was responsible for the increase in plasma palmitate concentration. Basal palmitate clear-

ance of  $1.20\pm0.13$  liters/min was essentially unaltered at any measured p[E]<sub>ss</sub> level (Fig. 4).

Plasma thresholds for increments in blood glucose, lactate, and  $\beta$ -hydroxybutyrate (data not shown) were in the 150–200-pg/ml range reported by us previously (22).





FIGURE 3 Change ( $\Delta$ ) in glycerol and palmitate concentrations at p[E]<sub>ss</sub> of 12–870 pg/ml. The  $\Delta$  palmitate and  $\Delta$  glycerol values were calculated from the basal and 30-min samples. The arrows indicate the estimated epinephrine thresholds.

FIGURE 4 Change ( $\Delta$ ) in inflow ( $R_a$ ) and outflow ( $R_d$ ) transport, and in clearance ( $R_c$ ) of plasma palmitate at p[E]<sub>ss</sub> from 12 to 870 pg/ml.  $\Delta R_a$  and  $\Delta R_c$  were calculated from the basal values and the peak values occurring at 20-30 min. The arrows indicate estimated thresholds for epinephrine effects.

	1.0			2.5			5.0	
B	I <sub>30</sub>	I <sub>60</sub>	В	I <sub>30</sub>	I <sub>60</sub>	В	I <sub>30</sub>	I <sub>60</sub>
116±8	$252 \pm 31$	188±21	$148 \pm 14$	388±43	$270 \pm 22$	$117 \pm 24$	396±79	304±46
$498 \pm 27$	$1092 \pm 119$	$806 \pm 77$	$612 \pm 34$	$1663 \pm 156$	$1286 \pm 95$	$479 \pm 93$	$1656 \pm 327$	$1273 \pm 189$
46±3	85±5	$64 \pm 10$	61±7	$126 \pm 21$	84±11	$50 \pm 19$	$125 \pm 43$	$72 \pm 28$
69±6	$118 \pm 21$	$153 \pm 34$	$100\pm7$	$258 \pm 16$	378±13	$101 \pm 22$	$250\pm68$	$350\pm94$

TABLE III (Continued)

# DISCUSSION

In regard to substrate and hormonal alterations produced by graded epinephrine infusions, the results of the present study agree well with those of our prior report (22). Basal and epinephrine-induced changes in glucose, insulin, lactate, glycerol, and  $\beta$ -hydroxybutyrate were virtually identical to those seen previously. Once again, we also saw no increase in plasma glucagon during epinephrine infusion, confirming the results of our previous study (22) but disagreeing with those of earlier investigations (45–47). However, our failure to see a plasma glucagon decrement with the modest hyperglycemia produced by the higher epinephrine infusion rates possibly reflects epinephrine's opposing effects on stimulating pancreatic alpha cell function (45–48).

Basal, postabsorptive palmitate and total FFA content varied substantially in the same individual, a phenomenon found by others (49). The use of the nonradioactive tracer [1-<sup>13</sup>C]palmitate, allowed us to repeatedly study basal palmitate flux, as well, in the same individual and confirmed that postabsorptive palmitate inflow transport varied between 13-28%(coefficient of variation), substantially greater than the less than 5% difference in basal glucose production found within the same individual by ourselves (22) and others (50), but consistent with the known direct relationship between plasma FFA levels and inflow transport (7, 44, 51-53) confirmed in the present work.

The current studies also support the hypothesis inherent in our previous investigation (22) that the lipolytic actions of epinephrine are manifest at lower plasma epinephrine levels than are its hyperglycemic and ketonemic effects. As before (22), the plasma epinephrine threshold for an increment in glycerol was in the 75-125-pg/ml range, and in the present study, a similar threshold was observed for increasing the plasma FFA level as well. This threshold contrasts with the somewhat higher plasma epinephrine values required to elevate plasma glucose, lactate, and  $\beta$ hydroxybutyrate. The kinetic studies carried out with  $[1-1^{3}C]$  palmitate confirmed that the increments in plasma FFA and glycerol seen at epinephrine concentrations >75–125 pg/ml were due to accelerated lipolysis and not decreased clearance, since palmitate R<sub>a</sub> increased within this range of epinephrine concentrations, but palmitate clearance was unaffected by epinephrine levels as high as 870 pg/ml. The latter finding, however, does not necessarily indicate that palmitate clearance is unaltered by changes in plasma palmitate content alone since, in the present study, plasma insulin levels also changed.

The absolute magnitude of the increase in palmitate R<sub>a</sub> is influenced by estimated pool size substituted in Steele's equations. Since virtually all circulating FFA are bound to albumin, we and others (44, 54) have used the plasma volume as the distribution space for calculating the FFA pool size. Although an extravascular albumin pool exists (55), this pool mixes very slowly (over an 8-h period in the dog) with intravascular albumin (55) and is unlikely to appreciably influence the FFA tracer distribution space in the 10min intervals between palmitate R<sub>a</sub> calculations. On the other hand, both Baker and Schotz (56) and Eaton et al. (37) have identified an extravascular FFA pool by compartmental analysis of pulse-injected [1-14C]palmitate. The exact size and nature of this pool are unknown. In the basal steady-state situation, the presence of this pool will have no effect on the reported palmitate Ra. However, omission of the extravascular FFA content in the nonsteady-state calculations used in the current work means that changes in palmitate inflow reported here should be regarded as minimal estimates. The magnitude of the variation in calculated palmitate inflow transport introduced by using different estimates of palmitate pool size can be appreciated by the following example. In the present studies, the average palmitate R<sub>a</sub> during the first 30 min of epinephrine infusion at  $5 \mu g/\min \cos 3.49 \mu \mod/kg \operatorname{permin}$ . If one assumed that the extravascular concentration of palmitate was the same as the plasma content (a likely

overestimation because of the low extravascular albumin levels) and that the tracer distributed throughout two-thirds of this extravascular pool during the 10-min sampling interval, the calculated average palmitate  $R_a$  would have been 4.08  $\mu$ mol/kg per min. The actual palmitate inflow rate almost certainly lies between these limits. The relative differences presented, of course, are unaffected.

The palmitate  $R_a$  showed a virtually immediate response to an elevation in plasma epinephrine, a long-appreciated phenomenon (7). The maximal palmitate  $R_a$  was achieved within 20–30 min and the peak palmitate concentration measured ~10 min later. Thereafter, plasma palmitate inflow transport and, consequently, palmitate content declined. This decrement appears to be due, in part, to the combined effects of hyperglycemia, per se (57), a slight increase in insulin values (secondary to hyperglycemia) at the higher epinephrine infusion rates and to the rise in lactate seen at higher  $p[E]_{ss}$ , since lactate is known to inhibit fat mobilization (54).

It is noteworthy that the increase in palmitate  $R_a$ occurs at plasma epinephrine values barely threefold the average basal value of 34±18 pg/ml of 60 normal subjects studied in our laboratory (58). This magnitude of epinephrine rise is only slightly greater than that achieved during guiet standing (59) and in the range found in subjects smoking cigarettes (60). Furthermore, the epinephrine threshold for its lipolytic effects is far below plasma epinephrine values encountered during exercise (61), myocardial infarction (62), diabetic ketoacidosis (63), and surgical procedures (22). Clearly, then, circulating epinephrine levels are more than sufficient to account for the lipolysis seen in these circumstances and long attributed to catecholamines. The effect of the circulating norepinephrine threshold on lipolysis was not addressed in the present study. Norepinephrine infusion rates of 4-5  $\mu$ g/min in man (64) and 0.05  $\mu$ g/kg per min in the dog (65) can elevate plasma FFA levels. We have shown (66) that these rates (the latter approximately equal to the former for a 70-kg subject) produce circulating norepinephrine levels in the range of 1,400-2,100pg/ml, at which level we also saw an increase in plasma glycerol. Although this norepinephrine range is reached during exercise (61), and with illnesses such as ketoacidosis (63) and myocardial infarction (67), it is rarely approached under ordinary physiological circumstances. Thus, under usual conditions, it appears that the lipolytic actions of norepinephrine may be limited to its long recognized local effector role as sympathetic neurotransmitter at nerve endings in adipose tissue.

We conclude that (a) small increments in plasma epinephrine can induce lipolysis, (b) lipid mobiliza-

tion is more sensitive than glucose production to increases in plasma epinephrine, and (c) circulating epinephrine is ~15-20-fold more potent than norepinephrine in promoting liposlysis.

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