Stimulation of Insulin Secretion by Infusion of Free Fatty Acids

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ABSTRACT The acute elevation of plasma free fatty acid (FFA) levels by direct infusion of sodium oleate into the plasma of conscious dogs was accompanied by the rapid onset of a 2- to 12-fold increase in plasma immunoreactive insulin, and, subsequently, a marked fall in plasma glucose, even in dogs receiving intravenous glucose throughout the infusion. The magnitude of both the insulin and glucose responses correlated with the mean FFA level during infusion. A large increase in plasma insulin and fall in glucose also occurred when glycerol was infused with oleate in order to simulate endogenous lipolysis more closely. Insulin levels in pancreaticoduodenal vein blood rose markedly during oleate infusion, while plasma ketone levels rose only slightly.

In contrast to the effects of oleate infusion, elevation of plasma FFA to correspondingly high levels by triolein ingestion and intravenous heparin produced only small increases in plasma insulin, which did not correlate well with the FFA level reached, and small increases in plasma glucose.

The results indicate that under certain conditions elevated FFA levels may be a potent stimulus of insulin secretion. This response is modified under other conditions such as during chylomicron removal under the influence of heparin. This effect may play a role in the regulation of lipolysis and ketone formation, but determination of the exact mechanism of FFA stimulation of the pancreas and its physiological significance will require further investigation.

Preliminary reports of part of this work have appeared (1) and were presented at the Annual Meeting of the Council on Arteriosclerosis, American Heart Association, Bal Harbour, Fla. 20 November 1968 (2).

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INTRODUCTION

Preliminary studies in this laboratory (3) showed that a marked decrease in blood glucose occurred during acute elevation of plasma free fatty acids (FFA) levels by infusion of sodium oleate directly into the plasma of conscious dogs. Subsequently, we found that a striking elevation of plasma insulin levels accompanied the glucose response (1). At that time, Seyffert and Madison (4) independently reported that the acute elevation of plasma FFA levels by intravenous infusion of triglyceride emulsion and heparin also was accompanied by a rise in plasma insulin and fall in glucose in anesthetized dogs with chronic portacaval shunts.

In contrast, Schalch and Kipnis (5) and Balasse and Ooms (6) have reported that elevation of plasma FFA by fat ingestion and intravenous heparin did not significantly affect basal insulin and glucose levels in man. In the present study, the effects of FFA infusion on insulin secretion and blood glucose levels were determined and then compared to those accompanying administration of a fat meal and heparin.

METHODS

FFA infusions. The method of elevating plasma FFA levels in dogs by direct infusion of FFA salts during continuous-flow centrifugation has been described in detail (7).

Mongrel dogs of both sexes were studied after an overnight fast. Except where noted anesthesia was not employed. Carotid artery blood was continuously separated into cells and plasma at a constant rate of 150–200 ml/min, and each component was returned to the dog. Sodium oleate 3% (106 μEq/ml) in 0.082 M saline was infused into the plasma line with a mean ratio of 7.74 μEq ($\pm$1.27 SD)/ml of plasma flow per min. Control periods of 60 min preceded and followed each oleate infusion. During these periods, normal saline was infused at the same rate (milliliters per minute) used for sodium oleate. Anticoagulation was maintained by constant infusion of a concentrated heparin-saline solution so as to deliver 2000 U of heparin in a volume of 25 ml/hr.

Pancreatic insulin secretion. Insulin levels in pancreaticoduodenal vein blood during oleate infusion were studied in two dogs. After a laparotomy under pentobarbital anesthesia, two polyethylene catheters (I.D. 1.2 mm) connected by a "T-fitting" were placed between the superior pancreaticoduodenal vein and the portal vein, respectively. Pancreatic blood flow, measured periodically by diverting blood flowing from the catheter into a graduated cylinder, did not vary by more than ±12%. The dogs were connected to the centrifuge by means of femoral artery–femoral vein shunts. All other conditions were the same as those for oleate infusions in the unanesthetized dogs.

Fat-feeding studies. Six unanesthetized dogs were connected to the IBM centrifuge as for oleate infusions. Arterial blood was separated into cells and plasma at the rate of 70 ml/min, and both fractions were returned to the dogs continuously. During a 6 hr control period, a glucose solution of 10 g/100 ml was infused at 1 ml/min; then each dog ingested 200 g of triolein[1] during 10-15 min, and the glucose infusion and centrifugation were continued for another 6 hr. Heparin was also administered continuously at the rate of 2000 U/hr throughout the study. The fat meal was omitted in eight additional dogs that served as 12-hr controls.

Chemical analyses. Blood was sampled from the arterial shunt line and pancreaticoduodenal vein at 10- to 15-min intervals and chilled immediately to 4°C to prevent lipolysis in vitro. After centrifugation at 4°C, the plasma was either analyzed at once or rapidly frozen for later determination. Measurement of plasma glucose and FFA were as noted in the previous paper (7).

Plasma immunoreactive insulin was assayed by a modification (8) of the double-antibody method of Morgan and Lazarow (9), using a crystalline pork insulin standard,[2] pork insulin-^{125}I,[3] guinea pig anti-pork insulin serum,[4] which reacted identically with pork and dog insulin, and rabbit anti-guinea pig γ-globulin serum.[4] Insulin values are the means of duplicate analyses, which agreed within 10%. In a representative assay of 51 pairs of duplicate samples (range: 2–116 μU/ml), the standard deviation between duplicates was 1.6 μU/ml (10). Consistency of assay results was maintained by performing all assays with aliquots of the same insulin standard and anti-insulin serum. The addition of sodium oleate to plasma in vitro did not affect the assay of either glucose or insulin.

Plasma ketone bodies were assayed by the method of Chernick (11). Differences between values were tested for significance by Student's *t* test.

RESULTS

Effect of sodium oleate infusion. When oleate was infused at high rates, plasma insulin rose to very high levels within 10 min after the start of the infusion. Blood glucose usually began to fall 10–20 min thereafter, and marked hypoglycemia developed (Table I). Lower elevations of FFA produced similar but smaller effects (Fig. 1). The fall in blood glucose, however, was not equally rapid in all experiments and was particularly slow in two dogs infused under pentobarbital anesthesia. As glucose levels fell during oleate infusion, plasma insulin often declined somewhat from initial peak levels. At the end of each FFA infusion (duration: 45–150 min), plasma FFA and insulin rapidly dropped to pre-infusion levels, and plasma glucose rose toward normal.

Although in most experiments the maximum rise in insulin and fall in glucose occurred at 15 and 45 min after onset of the oleate infusion, respectively, some variation in the time of maximum response was apparent. Thus, for purposes of ready comparison of the magnitude of the responses in many experiments, the mean of five control values was compared with the mean of three successive samples obtained over the 30 min infusion period during which there was a maximum response for insulin and glucose, respectively. By these criteria, a significant rise in plasma insulin and fall in glucose occurred in almost all experiments (Table I).

Over the range of 1.0–2.0 μEq/ml, the magnitude of the rise in plasma insulin and fall in glucose correlated with the mean FFA level during infusion (Fig. 2). A minimum stimulatory level was not determined. Simultaneous infusion of glycerol[5] with oleate (1:3 molar ratio), in order to simulate endogenous lipolysis more closely, did not significantly alter the insulin and glucose responses.

Effect of oleate infusion on animals receiving a continuous glucose infusion. In four dogs infused with oleate (mean rate: 39 μEq/kg per min) while receiving a continuous glucose infusion (100 mg/min), the time course and magnitude of the changes in FFA, insulin, and glucose were similar to those found in dogs not given glucose. These responses also were not affected by simultaneous infusion of glycerol with oleate (Fig. 3). In one study, however (Experiment 16), oleate infusion at 80 μEq/kg per min produced a striking dissociation of the insulin and glucose responses. Mean plasma insulin rose to 725 μU/ml (SEM ±99), the highest level reached during any oleate infusion, but blood glucose declined very slowly.[6]

To determine whether the insulin and glucose responses were due to acid-base changes with FFA infusion, 3% sodium bicarbonate was infused at 140 μEq/kg per min into a dog receiving a continuous glucose infusion (100 mg/min) during centrifugation. In contrast to the effects of sodium oleate infusion, infusion of sodium bicarbonate produced a moderate fall, rather than a rise, in plasma insulin, and a slight fall in plasma glucose (14 mg/100 ml) after 45 min of infusion. The gradual fall in glucose seen in this experiment is a response that

[1] Nutritional Biochemicals Corporation, Cleveland, Ohio.
[2] Kindly supplied by Dr. Otto K. Behrens, Eli Lilly & Co. Research Laboratories, Indianapolis, Ind.
[3] ISO/Serve Division, Cambridge Nuclear Corp., Cambridge, Mass.
[4] Arnel Products Co., New York.
[5] Glycerin, A. C. S. grade, Allied Chemical Corp., Morristown, N. J.
[6] Tables containing the data of the individual animals have been deposited with The American Documentation Center.

TABLE I

*Summary of Effects of Oleate Infusion on Plasma FFA, Glucose, and Insulin**

Exp. No.	Dog	Infusion rate	Mean plasma FFA			Mean plasma glucose			P value	Mean plasma insulin			P value
			Control	Infusion	Δ	Control	Infusion	Δ		Control	Infusion	Δ	
		μEq/kg per min		*μEq/ml*			*mg/100 ml*				*μU/ml*		
High elevation of plasma FFA													
1	A	58.3	0.59 ±0.02	1.80 ±0.12	1.21 ±0.12	109 ±4	71 ±8	38 ±9	<0.01	7 ±1	31 ±5	24 ±5	<0.01
2	B	59.0	0.87 ±0.05	1.94 ±0.08	1.07 ±0.11	106 ±2	52 ±2	54 ±3	<0.01	6 ±1	72 ±55	66 ±56	NS
3	C	64.8	0.66 ±0.05	1.87 ±0.08	1.21 ±0.09	111 ±6	66 ±5	45 ±8	<0.01	8 ±3	64 ±3	56 ±4	<0.01
4	C	43.3	0.65 ±0.08	1.62 ±0.10	0.97 ±0.12	121 ±4	83 ±3	38 ±5	<0.01	26 ±5	115 ±25	89 ±25	<0.05
5	D	40.8‡	0.50 ±0.03	1.41 ±0.02	0.91 ±0.04	92 ±2	71 ±2	21 ±3	<0.01	6 ±1	49 ±13	43 ±13	<0.05
6	E	35.5§	0.36 ±0.03	1.57 ±0.03	1.21 ±0.04	123 ±2	95 ±1	28 ±3	<0.01	12 ±1	28 ±3	16 ±3	<0.01
7	D	29.5§	0.31 ±0.02	1.40 ±0.04	1.09 ±0.04	143 ±3	87 ±6	56 ±6	<0.01	14 ±3	43 ±5	29 ±6	<0.01
Mean ±SE		47.3 ±5.1	0.56 ±0.07	1.66 ±0.08	1.10 ±0.11	115 ±6	75 ±5	40 ±8		11 ±3	57 ±11	46 ±12	
Low elevation of plasma FFA													
8	F	37.2	0.56 ±0.03	1.05 ±0.07	0.49 ±0.07	106 ±3	90 ±6	16 ±7	NS	4 ±1	12 ±1	8 ±2	<0.01
9	D	36.0	0.41 ±0.01	0.99 ±0.05	0.58 ±0.05	131 ±3	99 ±0	32 ±3	<0.01	7 ±1	16 ±1	9 ±1	<0.01
10	F	24.8	0.57 ±0.04	0.98 ±0.03	0.41 ±0.05	106 ±1	98 ±1	8 ±1	<0.01	7 ±1	16 ±1	9 ±1	<0.01
11	G	19.4	0.55 ±0.05	1.07 ±0.05	0.52 ±0.07	114 ±2	103 ±2	11 ±3	<0.05	8 ±1	15 ±1	7 ±2	<0.01
Mean ±SE		29.4 ±4.3	0.52 ±0.04	1.02 ±0.02	0.50 ±0.04	114 ±6	97 ±3	17 ±6		7 ±1	15 ±1	8 ±1	
Simultaneous glucose infusion, *100 mg/min*													
12	H	32.1	0.34 ±0.01	1.33 ±0.04	0.99 ±0.04	128 ±3	103 ±1	25 ±4	<0.01	16 ±1	37 ±3	21 ±3	<0.01
13	I	45.7	0.34 ±0.04	2.02 ±0.07	1.68 ±0.09	139 ±2	65 ±2	74 ±3	<0.01	13 ±1	83 ±11	70 ±12	<0.01
14	J	34.8	0.37 ±0.03	1.42 ±0.03	1.05 ±0.05	153 ±3	99 ±4	54 ±5	<0.01	31 ±8	121 ±44	90 ±44	NS
15	K	43.6‡	0.45 ±0.04	1.67 ±0.04	1.22 ±0.05	135 ±5	93 ±1	42 ±5	<0.01	13 ±1	31 ±4	18 ±4	<0.01
16	L	80.0‡,‖	0.42 ±0.01	1.85 ±0.11	1.43 ±0.11	151 ±2	134 ±1	17 ±3	<0.01	39 ±2	725 ±99	686 ±99	<0.01
17	M	31.7¶	0.42 ±0.05	1.07 ±0.05	0.65 ±0.07	359 ±9	318 ±9	41 ±13	<0.05	76 ±1	156 ±22	80 ±22	<0.05
Mean ±SE		39.1 ±3.3	0.38 ±0.03	1.61 ±0.16	1.23 ±0.05	139 ±5	90 ±9	49 ±10		18 ±4	68 ±21	50 ±22	
Sodium Bicarbonate infusion, *140 μEq/kg per min*													
18	N	0	0.38 ±0.02	0.32 ±0.02	0.06 ±0.03	150 ±2	117 ±1	33 ±2	<0.01	40 ±4	21 ±3	−19 ±6	<0.05
Pancreatic vein study													
19	PV-1	19.6§	0.23 ±0.02	1.60 ±0.11	1.37 ±0.11	126 ±6	137 ±1	11 ±6	NS	FA 5 ±1	18 ±0	13 ±1	<0.01
										PV 147 ±32	399 ±47	252 ±57	<0.01
20	PV-2	36.5§	0.37 ±0.02	1.48 ±0.06	1.11 ±0.07	137 ±3	99 ±7	38 ±8	<0.01	FA 5 ±1	173 ±33	168 ±33	<0.01
										PV 55 ±6	3544 ±661	3489 ±661	<0.01

FA, femoral artery; PV, pancreaticoduodenal vein; NS, not significant; FFA, free fatty acids.

* Control values are means ±SEM of five separate determinations during the 1 hr of saline infusion which preceded each oleate infusion. FFA infusion values are means ±SEM of all FFA determinations during infusion, while glucose and insulin values are means ±SEM of three determinations during the 30 min infusion period of maximum response for glucose and insulin, respectively.

‡ Glycerol combined with oleate (1:3 molar ratio).

§ Dog anesthetized with pentobarbital (30 mg/kg).

‖ Extraordinary rate; values not included in group means.

¶ Glucose infusion at 500 mg/min; values not included in group means.

has often been observed in dogs receiving glucose infusions at a constant rate (12).

Effect on plasma ketones. Total ketone levels were measured in four dogs infused with oleate at high rates. Two of them received oleate during a continuous glucose infusion (K and L, Table I), and their ketone levels did not rise significantly, even when a plasma FFA level above 1.8 μEq/ml was maintained for over 75 min (experiment 16). In the other two dogs (B and C, Table I), ketone levels rose slightly during oleate infusion, but at the time of maximum insulin secretion did not exceed twice the control levels (0.27 mm/liter).[6] Moreover, throughout each oleate infusion studied, plasma ketones were always less than 20% of the high levels reported to stimulate insulin secretion in dogs (13, 14).

Effect of oleate infusion on pancreatic insulin secretion. In the two dogs studied, infusion of oleate produced a significant rise in pancreaticoduodenal vein insulin levels, and peripheral insulin levels mirrored those in the pancreatic effluent blood (Table II). At a high rate of oleate infusion, pancreaticoduodenal vein insulin levels increased to 80 times the control values (Fig. 4). In both experiments, plasma insulin declined somewhat from maximum levels before the end of the FFA infusion. Blood glucose declined less sharply in these experiments than during oleate infusion in unoperated dogs, perhaps due to metabolic consequences of surgery.

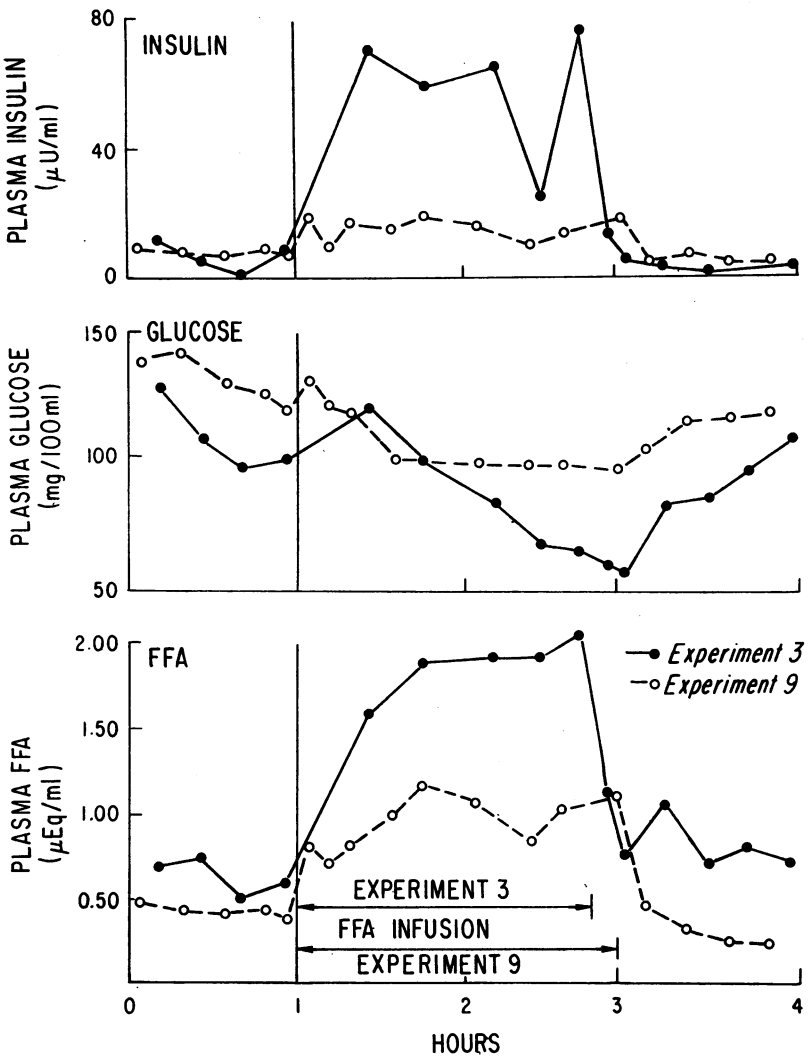

FIGURE 1 Comparison of the effect of sodium oleate infusion at high and low rates on plasma FFA, glucose, and insulin. After 1 hr of saline infusion, oleate was infused at 64.8 μEq/kg per min in experiment 3, and 36.0 μEq/kg per min in experiment 9.

Effect of fat feeding and heparin. After triolein ingestion and intravenous heparin, generally small increases in plasma insulin occurred, but they did not correlate well with the FFA levels reached, in contrast to the effects of oleate infusion. The rise in insulin was actually greater in the three dogs experiencing only a modest increase in FFA levels than in the three in which FFA rose to higher levels (above 1.50 μEq/ml) (Table III). Plasma glucose also rose slightly after the fat meal, but was not significantly greater than glucose levels in control dogs except in the 5th hr after fat ingestion (Fig. 5).

DISCUSSION

The results indicate that in dogs the acute elevation of plasma FFA levels by sodium oleate infusion is a potent stimulus of insulin secretion. Mean elevation of plasma FFA to 1.0 μEq/ml produced a 2-fold increase in peripheral plasma insulin concentration, and larger increases in FFA produced correspondingly higher insulin levels. The magnitude of the subsequent fall in blood glucose also correlated with the FFA level during infusion.

It is important to consider whether these responses could have resulted from any factor other than the elevated FFA level produced. As reported previously (7),

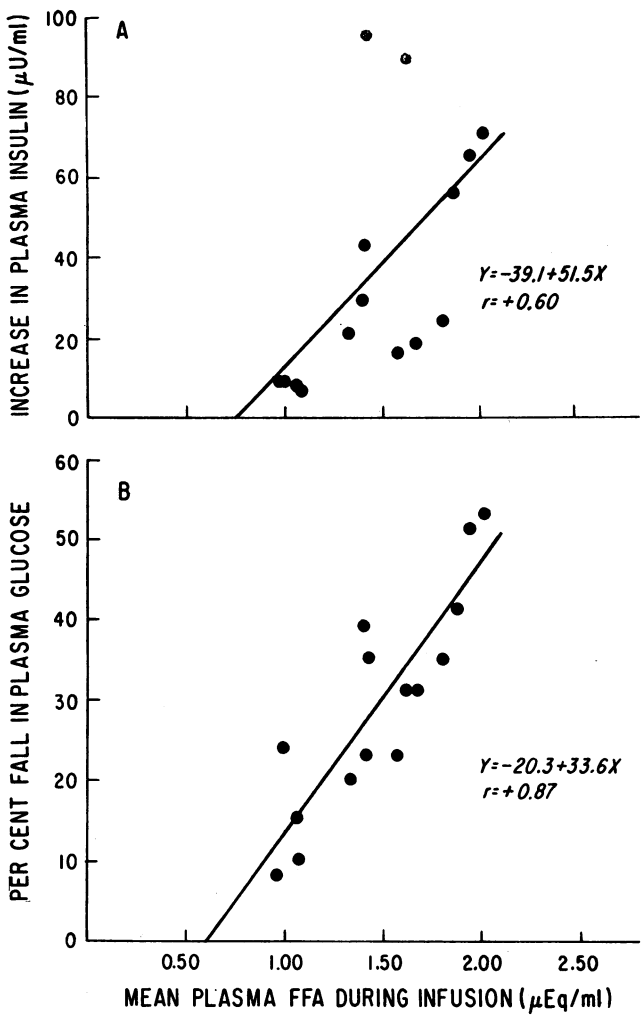

FIGURE 2 Regression analysis of the relation between mean plasma FFA during infusion and (A) increase in plasma insulin, and (B) percentage fall in plasma glucose, in experiments 1–15. Insulin and glucose responses were calculated from the differences between mean control levels and mean values during the 30 min infusion period of maximum response for insulin and glucose, respectively. In both cases, r was significant ($P < 0.02$ for A, $P < 0.01$ for B).

results of control studies of the metabolism of dogs subjected to continuous centrifugation make it unlikely that this process per se could account for the changes in insulin and glucose. In the present study, little change in plasma insulin or glucose occurred in the eight control dogs subjected to 12 hr of continuous centrifugation and heparinization, except for the gradual decline in blood glucose over several hours that is commonly observed during a constant glucose infusion (12). Moreover, no stimulation of insulin secretion was observed when the dogs were receiving heparin and saline during the 60 min control period that preceded and followed each oleate infusion. It seems unlikely that the entry of extracellular potassium into cells during the mild metabolic

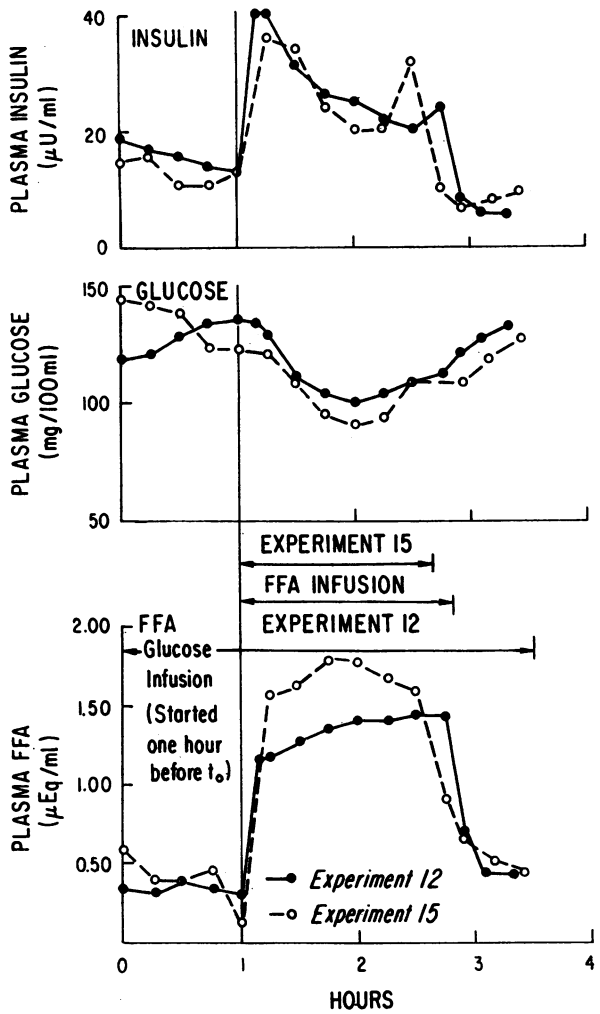

FIGURE 3 Comparison of the effect of sodium oleate infusion with and without glycerol on plasma FFA, glucose, and insulin during a constant glucose infusion (100 mg/min). Oleate was infused at 32.1 μEq/kg per min in experiment 12, and at 43.6 μEq/kg per min simultaneously with glycerol (14.5 μEq/kg per min) in experiment 15.

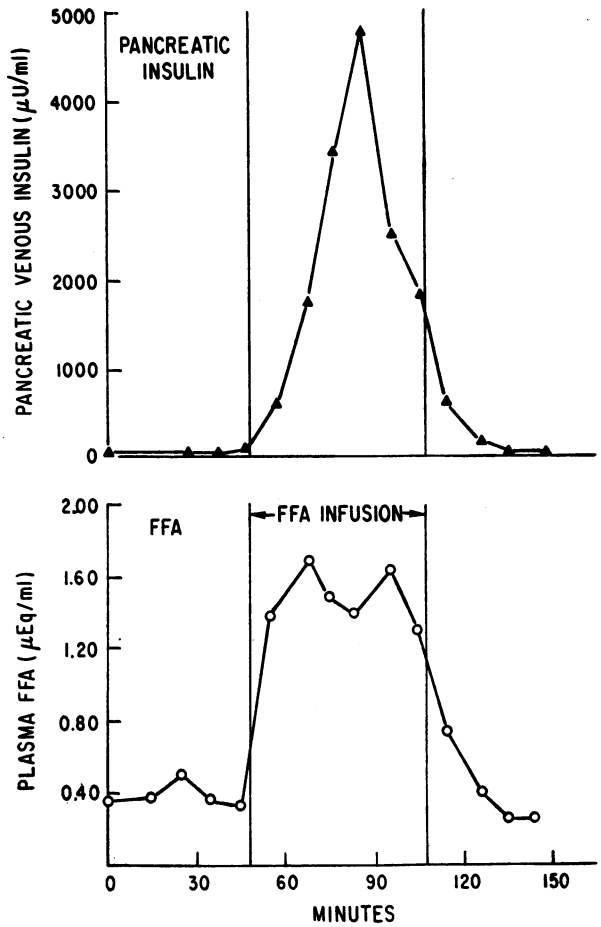

FIGURE 4 Effect of sodium oleate infusion (36.5 μEq/kg per min) on femoral arterial FFA and pancreaticoduodenal venous insulin.

alkalosis accompanying oleate infusion significantly affected insulin secretion, since no increase in plasma insulin was observed in the dog given a massive sodium bicarbonate infusion during centrifugation. It is also unlikely that sodium oleate evoked insulin release by stimulation of autonomic reflexes in the jugular vein or superior vena cava, since plasma insulin increased whether oleate was infused into the jugular vein or into the femoral vein.

Whether sodium oleate stimulates insulin release directly or whether the effect is indirect cannot be decided from these studies. It is unlikely that the insulin response was secondary to an increase in plasma ketone levels, since in most experiments they rose only slightly, if at all. Preliminary studies in our laboratory (15) have indicated that direct infusion of FFA into the pancreaticoduodenal artery of anesthetized dogs (at rates which did not raise systemic FFA) was in fact accompanied by a prompt and marked increase in pancreatic

TABLE II

Effects of Sodium Oleate Infusion on Plasma FFA, Glucose and Insulin, and Pancreaticoduodenal Venous Insulin

Exp. No.	Dog	Weight	Infusion rate	Measurement*	Preoleate control period, min				Sodium oleate infusion period, min									Postoleate control period, min		
					−45	−30	−15	0	10	20	30	40	50	55	60	70	100	10	25	40
		kg	*μEq/kg per min*																	
19	PV-1	15.9	18.6	FFA	0.22	0.23	0.19	0.23	1.28	1.36		1.89		1.70		1.88	1.83	0.80	0.32	0.28
				G	136	135	117	108	116	123		155		158		145	137	140	127	123
				IRI	2	4	4	6	3	8		11		16		15	18	15	9	9
				PV-IRI	254	186	75	101	93	140		359		493		344	253	117	197	222
20	PV-2	18.2	36.5	FFA	0.35	0.37	0.40	0.32	1.39	1.68	1.49	1.39	1.64		1.31			0.74	0.34	0.27
				G	143	128	136	144	140	139	139	144	155		136			129	106	86
				IRI	7	7	4	6	13	69	109	192	219		128			95	17	11
				PV-IRI	50		54	63	660	1733	3395	4756	2482		1814			640	144	65

* FFA, plasma FFA (μEq/ml); G, plasma glucose (mg/100 ml); IRI, plasma immunoreactive insulin (μU/ml); PV-IRI, pancreaticoduodenal venous insulin (μU/ml).

insulin levels. An apparently direct effect of FFA has also been reported recently by Malaisse and Malaisse-Lagae (16), who found that 0.5 mM palmitate stimulated insulin secretion in pieces of rat pancreas. On the other hand, Howell (cited by Montague and Taylor [17]), was not able to demonstrate an effect of palmitate on insulin secretion in rat pancreas slices, but the details of these experiments have yet to be published. The short-chain fatty acids butyrate and octanoate have been reported to stimulate insulin secretion both in vivo and in vitro (17–20).

Seyffert, Madison, Unger, and Barker (21) have reported that elevation of plasma FFA levels by intravenous infusion of a triglyceride emulsion and heparin stimulated insulin secretion in anesthetized dogs. They found that the elevation of plasma FFA from 390 to 1400 μEq/ml was accompanied by a rapid rise in plasma insulin from 45 to approximately 60 μU/ml in five dogs. Since the dogs were prepared with portacaval shunts, thus eliminating the appreciable hepatic uptake of insulin that normally occurs when pancreatic venous blood goes first to the liver (22, 23), the observed responses were probably maximal. Moreover, in contrast to the marked hypoglycemia seen at high FFA levels in the present experiments, after 90 min of triglyceride infusion Seyffert and Madison (4) observed (in 21 studies) a mean fall in blood sugar of only 14 mg/100 ml while plasma FFA rose from 0.54 to 1.83 μEq/ml. It seems likely that this more limited response was due in part to anesthesia, since in the present studies anesthesia was found to affect the time course of the glucose response to FFA infusion. Moreover, as discussed below, there may have been metabolic accompaniments of the indirect method used to raise FFA levels that also modulated the observed responses.

In the present studies, the individual contribution of hepatic glucose output and peripheral glucose utilization to the observed hypoglycemia cannot be assessed. It would appear that the magnitude and duration of the insulin response was such that both decreased hepatic glucose output and increased peripheral glucose utilization would occur (24). Nevertheless, it is quite possible that elevated FFA levels inhibited peripheral glucose utilization, but that hepatic glucose output was even more sharply reduced. Indeed, Seyffert and Madison calculated that the acute elevation of FFA by their method produced a 30% fall in peripheral glucose utilization and a 37% fall in hepatic glucose output. Moreover, an inhibitory effect of FFA on peripheral glucose utilization superimposed on the insulin effect in our studies could perhaps explain the dissociation of the insulin and glucose responses which occurred in some experiments. Thus the present studies are not incompatible with the glucose–fatty acid cycle hypothesis of Randle (25) who proposed that a reciprocal relationship exists between FFA and ketone metabolism on the one hand and the rate of peripheral glucose utilization on the other.

Although small increases in plasma insulin levels were noted after fat ingestion and intravenous heparin in some of the present studies, the increases did not correlate well with FFA level, as did the insulin response to oleate infusion. FFA levels, however, rose much more slowly in these fat-feeding experiments. It is also possible that in the hourly interval between samples, plasma insulin actually rose briefly to higher levels than those observed. Nevertheless, the present results appear similar to those in patients observed by Schalch and Kipnis (5) and Balasse and Ooms (6), who found that the acute elevation of FFA levels by injection of heparin after fat ingestion did not significantly affect basal insulin and glucose levels. Both groups, however, also found a significant decrease in the disappearance rate of glucose loads administered during FFA elevation.

TABLE III

Effects of Feeding Triolein on Plasma FFA, Glucose, and Insulin in Dogs Receiving Glucose Infusion (100 mg/min) during Continuous-Flow Centrifugation

Dog	Weight	Measurement	Hours after start of infusion												
			0	1	2	3	4	5	6*	7	8	9	10	11	12
	kg														
FF-21	19.5	FFA	1.51	0.73	0.37	0.43	0.41	0.38	0.37	0.37	0.58	0.63	0.83	0.89	0.74
		G	104	167	113	106	108	112	124	127	134	127	126	132	133
		IRI	7	5	8	7	8	9	7	6	7	9	11	19	17
FF-22	19.5	FFA	1.39	0.83	0.77	0.72	0.57	0.42	0.30	0.71	1.98	1.90	2.32	2.26	2.88
		G	93	168	202	197	174	151	123	107	124	121	140	153	149
		IRI	4	16	18	15	15	11	11	8	9	8	12	10	19
FF-23	18.3	FFA	0.98	0.50	0.42	0.39	0.39	0.36	0.43	0.74	0.80	0.98	0.99	0.95	1.05
		G	163	261	223	230	213	186	181	153	133	143	157	139	150
		IRI	12	49	35	39	45	46	24	43	45	54	73	55	56
FF-24	16.8	FFA	1.45	0.77	0.27	0.24	0.31	0.39	0.41	0.24	0.68	1.57	0.97	0.50	0.58
		G	113	173	193	170	163	188	188	144	122	125	136	138	126
		IRI	1	18	3	6	2	8	5	7	8	4	15	11	4
FF-25	20.0	FFA	1.70	0.94	0.58	0.78	0.57	0.62	0.62	0.53	0.93	0.70	0.54	0.51	0.59
		G	99	147	151	144	144	164	188	135	134	132	132	144	137
		IRI	1	9	12	9	9	5	6	14	9	11	17	17	13
FF-26	12.7	FFA	1.78	0.78	0.36	0.56	0.55	0.61	0.60	0.83	0.45	1.52	1.18	1.90	2.15
		G	99	194	170	147	151	171	152	147	158	144	137	133	133
		IRI	4	18	11	9	6	14	10	13	10	15	10	21	9
Mean ±SEM		FFA	1.47‡	0.76‡	0.46	0.52	0.47	0.46	0.46	0.57	0.90‡	1.22‡	1.14‡	1.17‡	1.33‡
		SEM	±0.12	±0.06	±0.07	±0.08	±0.05	±0.05	±0.05	±0.09	±0.23	±0.21	±0.25	±0.30	±0.39
		G	102	185	175	166	159	162	159	136	134	132	138	140‡	138
		SEM	±3	±16	±16	±18	±14	±11	±13	±7	±6	±4	±4	±3	±4
		IRI	5	19	15	14	14	16	11	15	15	17	23	22	20
		SEM	±2	±6	±5	±5	±6	±6	±3	±6	±6	±8	±10	±7	±8
Controls (n = 8)															
Mean ±SEM		FFA	0.90	0.52	0.38	0.35	0.38	0.35	0.36	0.39	0.37	0.37	0.35	0.35	0.34
		SEM	±0.11	±0.05	±0.03	±0.04	±0.05	±0.04	±0.04	±0.05	±0.04	±0.04	±0.03	±0.03	±0.02
		G	104	158	159	144	141	143	141	132	134	136	131	121	129
		SEM	±2	±13	±11	±10	±9	±8	±10	±7	±5	±7	±4	±6	±3
		IRI	6	15	16	15	13	14	15	13	13	15	14	12	13
		SEM	±2	±2	±3	±3	±2	±2	±2	±2	±2	±3	±3	±2	±3

FFA, plasma FFA (μEq/ml); G, glucose (mg/100 ml); IRI, immunoreactive insulin (μU/ml).
* 200 g triolein fed at start of 6th hr.
‡ Significantly greater ($P < 0.05$) than mean control value in corresponding period.

It is necessary to ask why the insulin and glucose responses apparently differed when FFA levels were raised by direct infusion as opposed to elevation by feeding fat in the presence of heparin. In the process of absorption of a fat meal, there is opportunity for a selective intestinal hormonal response (26). Under these conditions, there might, for example, be release of a hormone from the gut that inhibits insulin secretion, directly or indirectly. Another possibility is that there are metabolic accompaniments of the process of chylomicron removal beyond increasing intravascular FFA levels. For example, Ontko and Randle (27) have shown that chylomicrons inhibit glucose utilization in the perfused rat heart independent of any increase in FFA levels in the perfusate. One might speculate that chylomicrons have a direct metabolic effect on islet tissue tending to decrease insulin secretion. Another significant difference between the methods for FFA elevation is that when FFA levels were raised by oleate infusion there was no associated increase in lower glycerides and glycerol such as accompanies the action of lipoprotein lipase on chylomicrons. However, we observed no change in response during simultaneous infusion of glycerol with oleate. Finally, it is perhaps relevant that during infusion the rise in FFA was due primarily to an increment in oleate, instead of a mixture of fatty acids. However, we have recently found that infusion of linoleate also stimulated insulin secretion (15), and, as previously mentioned, palmitate has been found to stimulate insulin secretion in vitro (16). Moreover, the elevated FFA levels in the studies of Seyffert and Madison (4) presumably were due to increased levels of several fatty acids, reflecting the composition of the cotton seed oil emulsion which was infused.

Whether long-chain fatty acids play a significant role in the physiological regulation of insulin secretion remains to be established. During starvation, both plasma glucose and insulin levels have been reported to fall in

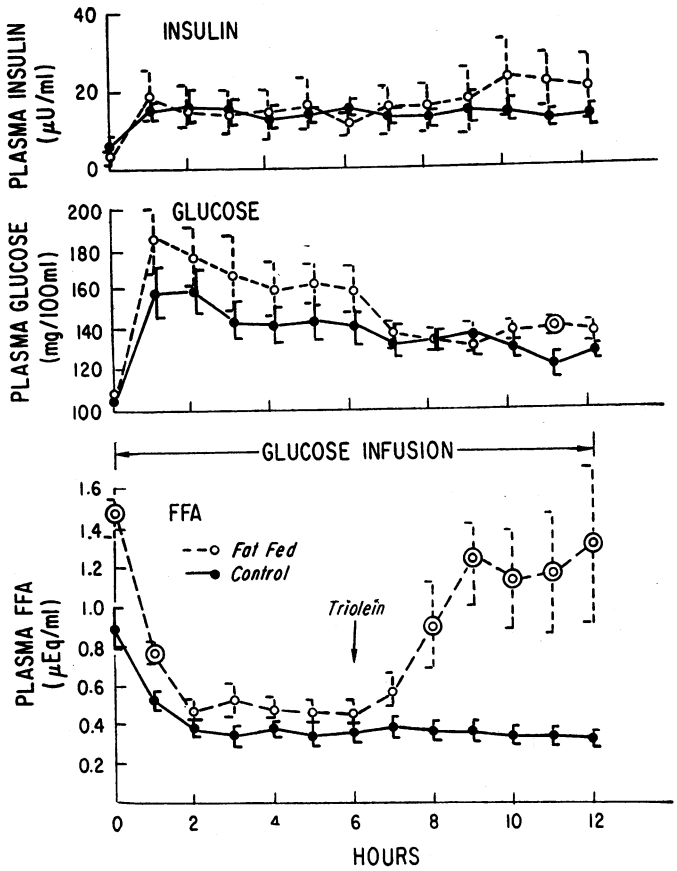

FIGURE 5 Effect of triolein feeding (200 g) on mean (±SEM) plasma FFA, glucose, and insulin in six dogs receiving a constant glucose infusion (100 mg/min) during 12 hr of continuous-flow centrifugation. Doubly circled values differed significantly ($P < 0.05$) from corresponding mean values of eight control dogs not fed triolein.

dogs and man (28). Thus, moderately elevated FFA levels do not appear to have a marked effect on insulin secretion in the metabolic setting of the starved organism. However, without the stimulus of FFA, insulin levels in starvation might be even lower than observed (4). Furthermore, a stimulatory effect of FFA on insulin could serve as a feedback control on the rate of lipolysis in adipose tissue (1). In addition, this FFA effect might modulate the inhibition of insulin secretion by epinephrine (29), one of the major physiological mediators of lipolysis.

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