

# Stimulation of Insulin Secretion by Long-Chain Free Fatty Acids

## A DIRECT PANCREATIC EFFECT

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**ABSTRACT** A continuous-flow centrifuge was used to infuse sodium salts of oleic, linoleic, lauric, or palmitic acid into the pancreatic artery of anesthetized dogs. In these regional perfusion studies there was no increase in FFA levels in the general circulation. Elevation of pancreatic FFA levels produced an immediate increase in pancreatic venous immunoreactive insulin (IRI). After 10 min of FFA infusion, IRI levels declined somewhat from the initial peak response but soon rose again to high levels which were then sustained until the infusion was terminated. All four long-chain FFA tested produced a similar biphasic IRI response. Clear-cut increases in IRI were associated with absolute FFA levels (measured in pancreaticoduodenal venous plasma) as low as 0.6–0.8  $\mu\text{eq/ml}$  and with increments over basal levels of as little as 0.4–0.5  $\mu\text{eq/ml}$ . At higher levels of FFA, absolute IRI levels in the pancreatic venous effluent exceeded 1,000  $\mu\text{U/ml}$  in some experiments and 5- to 10-fold increases over basal values were observed.

These studies indicate that long-chain FFA, in physiological concentrations, can markedly stimulate insulin secretion by a direct effect on the pancreas. The results lend support to the concept of insulin as a hormone that is importantly involved in regulating the metabolism of all three principal classes of metabolic substrates and whose release is in turn regulated by all of them. The relative importance and precise nature of its physiologic role in the regulation of lipolysis, lipid deposition, and ketone body formation remains to be established.

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*Received for publication 11 August 1972 and in revised form 12 March 1973.*

## INTRODUCTION

We have previously reported that the acute elevation of plasma free fatty acid (FFA) levels by systemic infusion of sodium oleate into conscious dogs was accompanied by a marked increase in immunoreactive insulin (IRI) and a fall in glucose levels in plasma (1). Seyffert and Madison reported similar effects accompanying the elevation of FFA levels produced by infusion of a triglyceride emulsion and injection of heparin into anesthetized dogs with chronic portacaval shunts (2). Sanbar, Evans, Lin, and Hetenyi demonstrated a hypoglycemic effect of octanoate in dogs with increase in plasma insulin levels (3). These studies do not establish whether the elevated FFA levels stimulate insulin release in vivo by a direct effect on the pancreas or whether the effect is indirect (e.g., secondary to metabolic effects in the periphery, effects via other endocrine systems or effects on the nervous system). There is some evidence that FFA may stimulate insulin secretion directly in vitro (4, 5) but other investigators have reported negative results (6, 7). In any case in order to establish physiologic significance it is still necessary to demonstrate effectiveness of FFA in vivo. In the present study, we have examined the nature of the insulin response accompanying infusion of long-chain FFA directly into the pancreatic artery of anesthetized dogs. These infusions raised intrapancreatic FFA levels without significantly increasing plasma FFA concentrations peripherally. We have also examined the sensitivity of the response and compared responses to four different fatty acids.

## METHODS

The general method for systemic infusion of FFA salts into mongrel dogs using a continuous-flow blood centrifuge

has been described in detail (8). For intrapancreatic infusion it was modified as follows. After an overnight fast, each dog was anesthetized with sodium pentobarbital (30 mg/kg) and connected to a mechanical respirator. The pancreas was exposed by a mid-line laparotomy and the superior pancreaticoduodenal artery and vein were isolated. The animal was then given 5,000 U of heparin intravenously and connected by carotid artery-jugular vein shunts to the continuous-flow centrifuge<sup>1</sup> as for systemic FFA infusion.

Carotid artery blood was continuously separated into cells and plasma, initially at 100 ml/min. These components emerged from the centrifuge via separate lines, were recombined, and returned continuously to the dog via the jugular vein.

After a 15 min stabilization period on the centrifuge, the pancreaticoduodenal artery was cannulated with a medium Bard-Deseret Intracath, and two cannulas, connected by a T-fitting, were inserted into the pancreaticoduodenal vein. Thus, except during sampling, blood flowed normally from the pancreatic vein into the portal vein. The return line from the centrifuge was then switched from the jugular vein to the pancreaticoduodenal artery and blood flow was reduced to a constant rate of 16–24 ml/min. The separated cells and plasma were recombined and infused into the pancreaticoduodenal artery at ambient arterial pressure (100–140 mm Hg) at 37°C.

Sodium salts of oleic, linoleic and lauric acids were prepared as previously described for oleate (8) and diluted to 0.5–1% with normal saline (final pH 9.4). They were then infused into the plasma line at constant rates of 0.8–1.4 ml/min (1.4–2.5  $\mu$ eq/kg per min), for 30 min. The concentrated FFA solutions did not dilute the carotid artery blood perfusing the pancreas by more than 6%, and did not significantly alter its pH. During control periods of 20 min which preceded and followed each FFA infusion, normal saline was infused into the plasma line at the same rate (ml/min) used for the FFA. Sodium palmitate for infusion was prepared by mixing equal volumes of 0.25–0.50% sodium palmitate (in 0.85% saline) and the dog's own plasma, which had been quickly derived from the plasma line of the centrifuge during the stabilization period. In two experiments, this mixture and the control mixture—which consisted of equal volumes of saline and the same dog's plasma—were enriched with canine serum albumin (Fraction V)<sup>2</sup> 3–4.5 g/100 ml. Infusion of Krebs-Ringer bicarbonate solution, pH 9.4, at 1.0 ml/min had no significant effect on insulin secretion. Pancreatic venous blood flow remained constant ( $\pm 15\%$ ) during each experiment.

Methylene blue dye infusion after each experiment revealed that a small amount of infusion mixture perfused the adjacent duodenum. To exclude the possibility that the concomitant perfusion of the duodenum affected the insulin response to intrapancreatic FFA infusion, an experiment was carried out in which sodium oleate was infused exclusively into a small area of the tail of the pancreas via the splenic artery, according to the technique of Goetz, Maney, and Greenberg (9).

Blood was sampled from the carotid and pancreaticoduodenal arteries and from the pancreaticoduodenal vein. It was chilled immediately to 4°C and analyzed for plasma FFA, IRI, glucose, and total ketones by methods previously described (1).

<sup>1</sup> Available as the Aminco Celltrifuge, American Instrument Co., Silver Spring, Md.

<sup>2</sup> Pentex, Inc., Kankakee, Ill.

## RESULTS

The regional infusion employed raised FFA levels in the pancreatic vein to values two to six times basal. Peak concentrations ranged from 0.88 to 2.55  $\mu$ eq/ml (mean 1.41). These elevations in local FFA concentration were effected by relatively low FFA infusion rates, ranging from 1.05 to 2.47  $\mu$ eq/min per kg. Since plasma FFA turnover in dogs is approximately 25  $\mu$ eq/min per kg, little or no change in systemic FFA levels would be expected. Carotid artery FFA concentrations were measured serially in all experiments; values during FFA infusion differed only insignificantly from pre- and post-infusion control values. Results from four representative experiments are shown in Table I. Again, because the FFA infusion rate was low on a whole body basis, no significant ketonemia was to be expected and spot checks verified this. Serial determinations were made in two studies and these results are shown in Table I (dogs 70 and 71).

When infusion of FFA was started, pancreatic vein FFA levels rose immediately to a new steady-state value and remained elevated until the end of the infusion (Figs. 1–3 and Table I). Within 2–5 min after the start of the infusion (the earliest samplings taken), pancreatic IRI began to rise and reached a peak of three to six times control levels at 5–10 min. Thereafter, IRI declined somewhat but still remained markedly elevated above control levels until the end of the infusion. Often a second peak in the insulin response curve was apparent after 20–30 min of infusion (Figs. 2 and 3 and Table I). When the infusion was terminated, pancreatic FFA and IRI levels fell quickly to control values.

The magnitude of the peak IRI response correlated roughly with the mean pancreatic FFA level reached during infusion. This is concordant with previous results using systemic infusion of FFA (1). All four long-chain FFA tested appeared to be approximately equipotent in stimulating insulin secretion although minor quantitative differences cannot be ruled out without further study. Even modest elevation of pancreatic FFA levels, to values of 1.1  $\mu$ eq/ml or less, produced three- to fourfold increases in pancreatic IRI (Fig. 3; Table I, dogs 75, 89, 70, 86, 88, 90). No attempt was made to establish a threshold for the FFA effect, which may depend on the initial level as well as the magnitude of the increment.

Insulin levels in the peripheral blood paralleled those in the pancreatic vein but of course at much lower values. Still, plasma glucose levels fell more gradually and less strikingly than might have been expected in view of the IRI response. This may possibly be due to the metabolic accompaniments (e.g., adrenocortical and adrenomedullary responses) of the anesthesia and surgery (10) since the hypoglycemic effect in conscious animals was more consistent and more marked (1).

TABLE I  
Effects of Direct Infusion of Fatty Acid Anions into the Superior Pancreatic Artery on Systemic and Pancreatic FFA, Glucose, and Insulin Levels in Anesthetized Dogs

Dog no.	Weight kg	FFA anion infused	Infusion rate $\mu\text{eq/kg/min}$	Pancreatic vein blood flow ml/min	Measurement	Pre-FFA control period (min)						Post-FFA control period (min)							
						FFA infusion period (min)						FFA infusion period (min)							
						-20	-10	0	2	5	10	15	20	30	+5	+10	+20		
75	18.3	1/3 Laurate	1.43	24	PV-F	0.45	0.46	0.47	—	0.94	1.09	1.12	1.12	1.16	0.71	0.48	0.37		
						PV-G	92	94	97	—	88	83	86	84	86	84	90	90	
						PV-I	279	99	179	—	898	529	783	920	920	920	405	363	
89	16.7	1/3 Laurate	1.64	32	PV-F	0.24	0.22	0.21	—	0.62	0.88	0.83	0.83	0.77	0.30	0.22	0.19		
						PV-G	109	113	113	—	106	106	99	93	—	91	102	114	
						PV-I	61	65	95	—	225	301	128	331	328	93	73	76	
77	15.9	1% Oleate	1.78	24	PV-F	0.65	0.55	0.51	—	1.20	1.29	1.31	1.28	1.49	0.47	0.40	0.34		
						PV-G	184	171	168	—	168	160	158	168	159	165	162	159	
						PV-I	42	53	65	—	134	161	192	201	323	85	56	64	
85	17.5	1% Oleate	2.02	24	PV-F	0.68	0.72	0.70	—	2.16	2.13	2.19	2.55	2.46	1.00	0.77	0.68		
						PV-G	73	72	79	—	74	77	75	68	68	59	69	59	
						PV-I	140	120	131	—	2,260	967	940	972	907	628	477	280	
68	20.0	1/3 Linoleate	1.87	24	SA-F	0.65	0.77	0.75	—	0.83	—	0.89	0.80	0.79	0.67	0.69	0.62		
						SA-G	86	81	88	—	91	—	86	85	71	70	74	66	
						SA-I	15	10	12	—	135	—	59	63	36	31	30	21	
70	14.1	1/3 Linoleate	2.47	18	PV-F	0.51	0.45	0.43	—	1.37	1.75	1.64	1.44	—	0.76	0.49	0.43		
						PV-G	108	105	106	—	109	110	112	112	—	127	127	131	
						PV-I	163	262	385	—	886	642	505	639	—	306	241	242	
71	19.1	1% Linoleate	2.15	24	PV-F	0.23	0.17	0.21	—	1.11	1.19	1.17	1.27	1.18	0.39	0.47	0.36		
						PV-G	113	111	115	—	116	122	127	132	159	180	192	204	
						PV-I	—	205	249	—	1,117	1,380	551	737	853	385	312	258	
86	18.6	1/3 Palmitate	1.05	36	PV-K	0.18	0.17	0.17	—	0.18	0.17	0.25	0.22	0.24	0.26	—	0.21		
						SA-F	0.22	0.23	—	—	0.25	0.29	—	0.36	0.43	0.37	0.39	—	
						SA-G	133	146	—	—	167	202	—	210	263	267	290	—	
88	17.5	1/3 Palmitate in 4.5% CSA	1.39	34	SA-I	6	13	—	—	—	18	27	—	46	55	44	45		
						PV-F	0.32	0.27	0.25	—	0.65	0.77	0.65	0.78	0.95	0.41	0.30	0.25	
						PV-G	108	122	123	—	115	108	107	100	95	84	95	79	
90	19.1	1/3 Palmitate in 3% CSA	1.27	30	PV-I	190	175	180	—	440	350	590	540	549	371	227	138		
						SA-F	0.31	0.23	0.24	—	0.22	0.21	0.18	0.34	0.25	0.21	0.21	0.17	
						SA-G	133	130	136	—	122	119	115	115	101	89	98	88	
91	Infusion via splenic artery	1/3 Oleate	1.58	2.0	PV-F	16	8	13	—	18	23	22	20	21	22	17	21		
						PV-F	0.39	0.34	0.30	—	0.99	1.19	—	1.06	1.10	1.09	0.29	0.20	0.18
						PV-G	118	111	118	—	115	111	—	107	109	113	107	105	104
91	Infusion via splenic artery	1/3 Oleate	1.58	2.0	PV-I	95	49	81	—	196	315	—	194	300	273	179	127		
						PV-F	0.33	0.34	0.38	—	0.52	1.04	0.96	1.04	0.87	0.23	0.22	0.22	
						PV-G	96	102	113	—	115	109	103	103	107	95	85	98	
91	Infusion via splenic artery	1/3 Oleate	1.58	2.0	PV-I	171	150	168	—	285	848	410	749	698	222	133	110		
						PV-F	0.38	0.29	0.31	—	0.48	1.16	—	1.18	1.29	0.57	0.29	0.19	
						PV-G	114	115	103	—	109	122	—	130	137	135	132	—	
91	Infusion via splenic artery	1/3 Oleate	1.58	2.0	PV-I	2,245	2,198	2,200	—	1,872	5,818	—	4,605	5,364	1,440	865	433		

PV, Pancreatic venous plasma; SA, carotid artery plasma; F, FFA ( $\mu\text{eq/ml}$ ); G, glucose ( $\text{mg}/100\text{ ml}$ ); I, immunoreactive insulin ( $\mu\text{U/ml}$ ); CSA, canine serum albumin; K, total ketone bodies (mM).

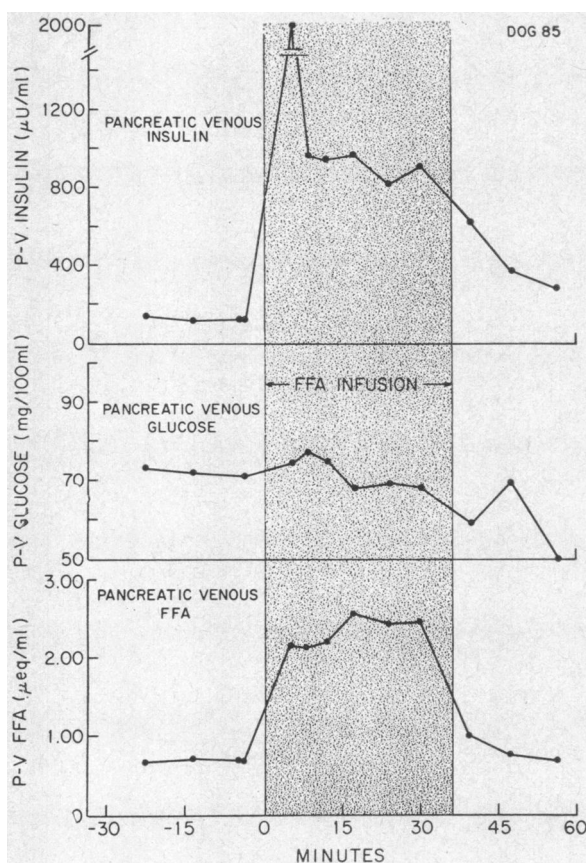


FIGURE 1 Effect of infusion of sodium oleate ( $2.02 \mu\text{eq/kg}$  per min) into the superior pancreaticoduodenal artery on pancreatic venous FFA, glucose, and IRI.

When sodium oleate was infused into the splenic artery branch supplying the tail of the pancreas, in order to prevent FFA-rich blood from reaching any other abdominal viscera, marked stimulation of insulin secretion also occurred (Table I, last line). The low blood flow in this study reflects the small portion of pancreas perfused. IRI values were high initially but doubled during FFA infusion.

### DISCUSSION

These results demonstrate for the first time in vivo that long-chain FFA, at concentrations within the physiologic range, can directly stimulate insulin secretion. Elevation of pancreatic FFA levels even to values less than  $1.0 \mu\text{eq/ml}$  produced a marked increase in IRI level. The saturated and unsaturated long-chain FFA tested appeared to be about equally potent in stimulating insulin secretion. Moreover, the same immediate insulin response was observed whether FFA were infused into the body of the pancreas or into the tail. The latter procedure excludes blood flow to all other viscera (9) and eliminates

the possibility that the observed insulin responses were secondary to FFA-stimulated release of enteric hormones known to stimulate insulin secretion (11). Since only one study was done in this manner, this possibility cannot be ruled out but in view of the limited area of duodenum receiving FFA-enriched blood the possibility seems remote. Since peripheral arterial levels of FFA were not elevated, effects involving the action of FFA on endocrine organs, the central nervous system or the autonomic nervous system with secondary effects on the pancreas are ruled out. Thus, it is concluded that the insulin response is a direct effect of FFA on the pancreatic beta cell.

Malaisse has reported that  $0.5 \text{ mM}$  palmitate stimulated IRI release from pieces of rat pancreas (4), while others have shown that the medium-chain FFA octanoate can stimulate IRI release both in vitro and in vivo (3, 5). However, Howell (cited in reference 6) was unable to demonstrate an effect of palmitate on rat islets, nor could Pi-Sunyer find an effect of  $3 \text{ mM}$  octanoate or oleate on insulin secretion from pieces of rat or rabbit pancreas incubated in vitro (7). Moreover, in preliminary experiments in which a continuous-flow centrifuge was not used to perfuse the pancreas, we were unable to stimulate insulin secretion by merely infusing FFA-

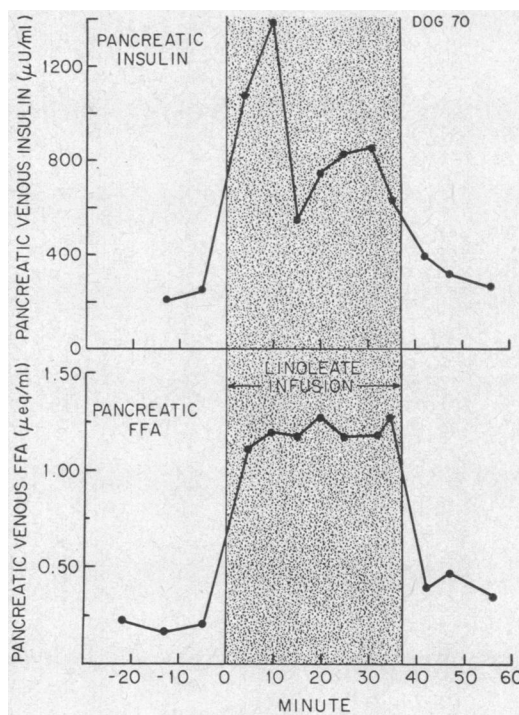


FIGURE 2 Effect of infusion of sodium linoleate ( $2.47 \mu\text{eq/kg}$  per min) into the superior pancreaticoduodenal artery on pancreatic venous FFA and IRI.

albumin mixtures into the pancreatic artery.<sup>3</sup> These negative results probably demonstrate the difficulty in preserving the physiologic milieu of the beta cell while concomitantly trying to elevate its FFA level.

Systemic glucose levels fell much less in the present studies than in previous studies using systemic FFA infusion into conscious animals (1) even though systemic insulin levels rose in some cases to values of 20–25  $\mu\text{U}/\text{ml}$ . The possibility that the proinsulin to insulin ratio was increased should be considered but since the stimulus (elevated arterial FFA levels) was the same in both sets of experiments this is unlikely to explain the difference. A decrease in insulin sensitivity secondary to surgery and anesthesia may be enough to account for the dampened glucose response (10).

The present studies extend the evidence that substrates derived from each of the three principal classes of metabolic fuels—carbohydrates, proteins, and fats—are capable of stimulating insulin release (12). Clearly insulin is not to be considered merely a hormone regulating carbohydrate utilization and storage, but rather a hormone broadly involved in regulation of and regulation by an array of metabolic fuels (13). The present studies also show that long-chain FFA are capable of producing a biphasic insulin response resembling that previously demonstrated for glucose and possibly amino acids (14, 15). Grodsky, Landahl, Curry, and Bennett (16) suggested that this response represents the early release of preformed insulin granules followed by a slower phase of release related to *de novo* synthesis of insulin. The infusion of gastrointestinal hormones such as secretin, however, produces only uniphasic insulin responses (12, 17).

The exact mechanism by which long-chain FFA stimulate insulin secretion is unknown. Montague and Taylor (18) reported that octanoate and citrate increased the intracellular concentrations of glucose-6-phosphate and 6-phosphogluconate in isolated rat islets incubated for 30 min. They proposed that insulin release was promoted by enhancing oxidation of glucose-6-phosphate through this pathway. Matschinsky et al. (19), however, did not find an increase in glucose-6-phosphate or 6-phosphogluconate in rat islets exposed to glucose for less than 5 min although insulin release occurred within 1 min. They proposed that glucose plays a dual role in islet metabolism, i.e., it is both a direct chemical stimulant of the early phase of insulin secretion as well as a substrate promoting newly synthesized insulin secreted in the later phase. Long-chain FFA could conceivably also play such a dual role in islet metabolism, but the intracellular metabolism of FFA in islets remains to be elucidated. We did not detect significant conversion of FFA to ke-

<sup>3</sup> Crespín, S. R., W. B. Greenough, and D. Steinberg. Unpublished observations.

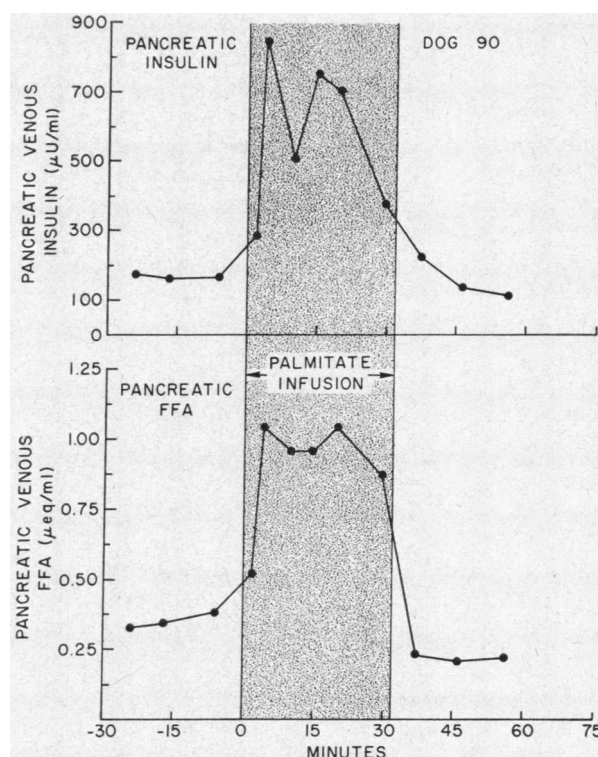


FIGURE 3 Effect of infusion of sodium palmitate (1.27  $\mu\text{eq}/\text{kg}$  per min) into the superior pancreaticoduodenal artery on pancreatic venous FFA and IRI.

tones, which are a known stimulus of insulin secretion in dogs (20, 21), in the pancreatic effluent blood. However, a selective conversion of FFA to ketones in only the endocrine and not in the exocrine pancreas would probably not be detected because the former comprises only a small fraction of the total pancreatic mass. Whether glucose, amino acids and fatty acids exert this effect via a common intermediate such as pyruvate or even ATP is not known (22).

In contrast to the marked stimulation of insulin secretion that accompanies systemic or intrapancreatic infusion of FFA in dogs (1, 2) elevation of long-chain FFA levels by oral fat and heparin does not cause a significant rise in plasma insulin in dogs (1) or man (23). The cause of this paradox is unknown but may be related metabolic effects occurring during fat digestion, absorption, or chylomicron removal. During chylomicron assimilation, small increments in insulin secretion could be undetected in peripheral plasma because of increased hepatic uptake. McCullough et al. (24) reported that stimulation of insulin secretion by medium-chain triglycerides could be demonstrated only in cirrhotics with evidence of portal-systemic communications. No increase in peripheral insulin was found in normal subjects. Thus,

a small increase in portal insulin levels caused by long-chain FFA could be masked in the periphery.

Whether long-chain FFA play a physiological role in the regulation of insulin secretion in man remains to be established. Such an effect could promote esterification of FFA during periods of feeding and inhibit lipolysis during fasting. The latter effect could represent a form of negative feedback control limiting hormone-stimulated lipolysis. It might also help maintain the low but detectable levels of insulin during fasting tending to prevent ketoacidosis (1, 2).

#### ACKNOWLEDGMENTS

The authors are grateful to Mr. David Boynton for his excellent technical assistance and to Dr. Sidney S. Chernick for his help in determinations of ketone body levels. This work was supported in part by U. S. Public Health Service Grant HL-12373.

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