Elevation of Plasma Fatty Acids by Ten-Hour Intralipid Infusion Has No Effect on Basal or Glucose-Stimulated Insulin Secretion in Normal Man

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There is controversy over the effect of free fatty acids (FFAs) on insulin secretion. Previous studies have shown opposite effects of short- and long-term exposure to elevated concentrations of FFAs. We studied 8 normal subjects (mean age, 30 years; mean body mass index, 23.4 kg/m^2) on 2 occasions. Each had a 10-hour overnight infusion of Intralipid 20% (Pharmacia, Milton Keynes, UK) with simultaneous infusion of heparin (0.4 U/kg body weight/min) or a control infusion of saline (150 mmol/L). Insulin secretion was assessed immediately after completion of the 10-hour infusion by an intravenous glucose tolerance test. Results were analyzed using paired *t* tests. Intralipid infusion caused an increase in plasma FFAs of more than 9-fold ($P < .01$), with a simultaneous increase in glycerol ($P < .01$) and hydroxybutyrate ($P < .01$). There was no difference in blood glucose concentrations during the infusion or intravenous glucose tolerance test. Similarly, insulin secretion was not significantly different during Intralipid infusion or in the intravenous glucose tolerance test (peak insulin achieved in glucose tolerance test, $P = .51$; total insulin secretion during intravenous glucose tolerance test, $P = .27$). In conclusion, after increasing plasma FFA concentrations over 9-fold during a 10-hour infusion of Intralipid and heparin, we found no difference in basal or glucose-stimulated insulin secretion.

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ELEVATED plasma concentrations of free fatty acids (FFAs) have effects on insulin action and may modify insulin secretion, and hence may play a role in the pathogenesis of type 2 diabetes mellitus. In the 1960s, Randle et al[1] promulgated the existence of a glucose–fatty acid cycle. Part of this cycle decrees that high plasma fatty acid levels result in increased fatty acid oxidation, which depresses glucose uptake into muscle and subsequent oxidative glucose metabolism. More recently, using clamp techniques, increasing FFA levels have been confirmed to impair insulin-induced inhibition of hepatic glucose production[2,3] and insulin-stimulated glucose uptake into muscle and thus cause insulin resistance.[3-6]

While impaired insulin action may be observed in cardiac and skeletal muscle, as well as adipocytes, only recently has attention focused on this relationship within the β cells of the pancreatic islets. Thus, the effect of elevated FFAs on insulin secretion is less clear. Early studies suggested that increased plasma FFAs stimulate insulin secretion.[7-9] Crespin et al[8] observed an increase in insulin secretion when sodium salts of fatty acids were infused directly into the pancreatic artery of anesthetized dogs. Latterly, Zhou and Grill[10,11] have reported the effects of elevated fatty acids on insulin secretion by perfused rat and human islets. They observed that elevated fatty acids initially enhance insulin secretion from islets, but continuous exposure to increased fatty acid concentrations leads to a significant reduction in insulin secretion.

Were this finding to be repeated in man in vivo, there would be important ramifications for the pathogenesis of type 2 diabetes and particularly the progression of the biochemical defect(s) with time. We have thus infused fatty acids, as Intralipid with heparin, into normal subjects for 10 hours to study the effects on basal and glucose-stimulated insulin secretion.

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SUBJECTS AND METHODS

Subjects

Eight Caucasian subjects (4 males) were recruited from personal contacts. The mean age was 30 years (range, 20 to 38) and the mean body mass index was 23.4 kg/m^2 (range, 19 to 26). All subjects were healthy and had no family history of diabetes. The female subjects were not pregnant and were not having unprotected sexual intercourse at the time of the study. Each subject received an information sheet detailing the aims and procedures of the study and had an opportunity to ask questions before providing informed consent to the study. The study was approved by the local Research Ethics Committee of the University Hospital Birmingham NHS Trust.

Protocol

Subjects attended for overnight infusion on 2 occasions separated by less than 8 weeks. Infusions were performed in random order and consisted of saline (150 mmol/L) 45 mL/h or Intralipid 20% (Pharmacia, Milton Keynes, UK) 45 mL/h. The Intralipid infusion was combined with an infusion of heparin (CP Pharmaceuticals, Wrexham, UK) 0.4 U/kg/min.

No alcohol was permitted during the 24 hours prior to the study. Subjects ate their usual evening meal before reporting at 9:20 PM. A retrograde cannula (20G Jelco; Ethicon, Pomezia, Italy) was inserted at 9:30 PM for blood sampling, and this hand was then placed in a hand-warming box at 55°C and the cannula was kept patent by a slow infusion of saline (150 mmol/L). A second cannula (18G Venflon; Ohmeda, Helsingborg, Sweden) was inserted in the contralateral arm for the infusion. After 20 minutes, basal samples were taken and the infusion was started. The infusion continued for 10 hours, with arterialized samples taken every 2 hours.

At the end of the infusion, a bolus of 50% glucose at a dose of 300 mg/kg body weight was injected over 3 minutes, followed by a rapid bolus of 20 mL saline. At the end of the bolus injection of glucose, a sample was taken (time 0) and then further samples were taken at 3, 5, 7, 10, 15, 20, 30, 40, 50, and 60 minutes. Subjects were then given breakfast and allowed to go home. All blood samples were assayed for

glucose, C-peptide, insulin, FFA, glycerol, 3-hydroxybutyrate, acetoacetate, and lactate.

Assays

Serum for FFA, insulin, and C-peptide determinations was stored at −20°C before analysis. Blood for glucose, glycerol, 3-hydroxybutyrate, acetoacetate, and lactate was added to ice-cold 0.77 mol/L perchloric acid upon collection, and the acid extracts were stored at −20°C. Acetoacetate measurements were performed within 48 hours of specimen collection.

Insulin and C-peptide levels were measured by radioimmunoassay (Pharmacia, Uppsala, Sweden, and Guildhay, Guildford, UK, respectively; internal quality-control limits, mean ± 2 SD). FFA concentrations were measured using a commercially available enzymatic system (NEFA-C; Wako, Neuss, Germany). Glucose, glycerol, 3-hydroxybutyrate, acetoacetate, and lactate levels were measured enzymatically using the procedures of Stappenbeck et al.[12]

Statistics

Data are expressed as the mean ± SEM. C-peptide, insulin, and ketone measurements were considered to be not normally distributed, and the results were thus logarithmically transformed before statistical analysis. The areas under the curve for the 10-hour infusion period and the following glucose tolerance test were compared for the control and the Intralipid and heparin infusion using the paired *t* test. Mean peak concentrations of insulin, C-peptide, and glucose achieved during the glucose tolerance test were compared using the paired *t* test. A significant result was required to have a *P* value less than .05.

RESULTS

Overnight Infusion

Plasma FFA and blood glycerol. During infusion of Intralipid plus heparin, both FFAs and glycerol increased significantly compared with infusion of saline (Fig 1 and Table 1). After 4 hours, the plasma FFA concentration reached a mean of 3.0 ± 0.38 mmol/L, and remained at this level until the end of the infusion ($P < .01$).

Blood glucose. Glucose concentrations decreased during the infusion period (Table 1). There was no statistical difference in glucose concentrations during this period between the two infusions ($P = .08$).

Blood ketones. Infusion of Intralipid plus heparin caused an increase in the concentration of both 3-hydroxybutyrate and acetoacetate (Fig 1 and Table 1). The increase in 3-hydroxybutyrate during Intralipid infusion was statistically significant ($P < .01$), whereas the change in acetoacetate failed to reach significance ($P = .08$).

Blood lactate. During both overnight infusions, lactate concentrations declined (Table 1). Lactate concentrations were higher during and after infusion with Intralipid plus heparin, but this was not a significant difference ($P = .26$).

Plasma insulin and C-peptide. Subjects were postprandial at the beginning of the infusion period, as reflected by the elevated concentrations of insulin and C-peptide. The concentra-

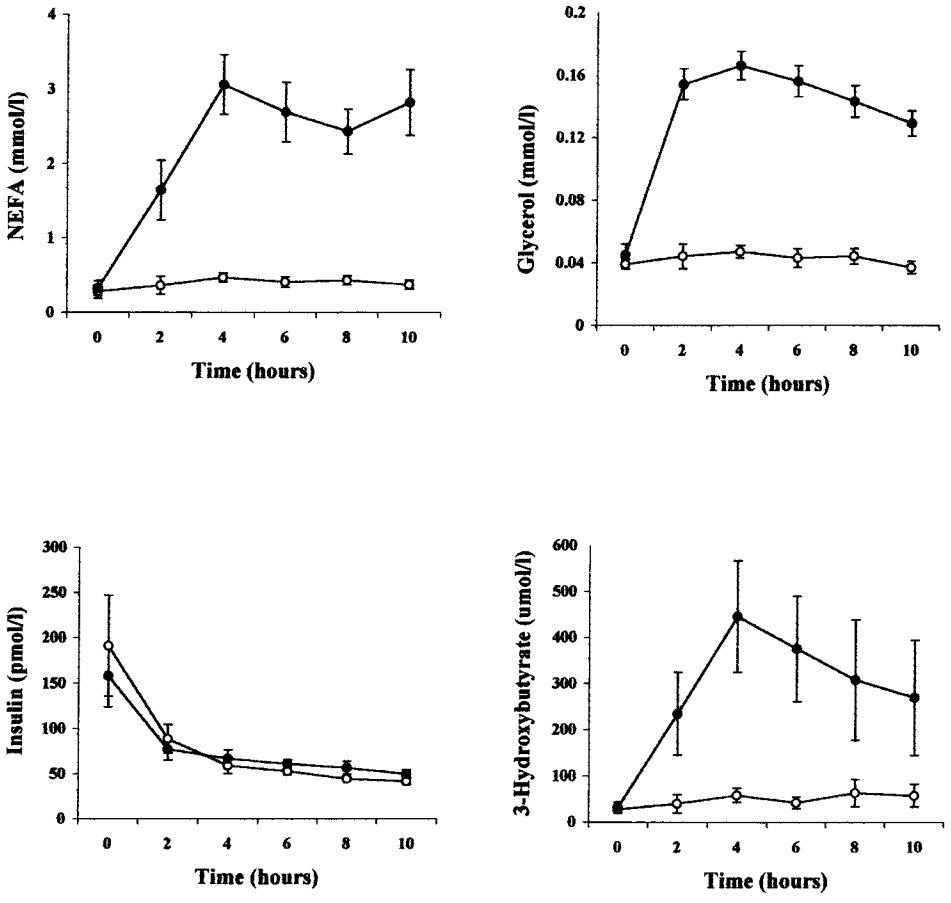

Fig 1. Hormone and metabolite concentrations (mean) during infusion of saline (○) or Intralipid plus heparin (●) in 8 normal subjects.

Table 1. Hormone and Metabolite Concentrations at the Start and End of 10-Hour Intravenous Infusion of Saline or Intralipid Plus Heparin in Eight Normal Subjects

Parameter	Start		End		P*
	Saline	Intralipid	Saline	Intralipid	
FFA (mmol/L)	0.28 ± 0.09	0.32 ± 0.10	0.37 ± 0.06	2.82 ± 0.45	<.01
Glycerol (mmol/L)	0.04 ± 0.003	0.05 ± 0.007	0.04 ± 0.005	0.13 ± 0.009	<.01
Glucose (mmol/L)	5.8 ± 0.2	5.7 ± 0.3	4.9 ± 0.3	4.6 ± 0.1	.08
Hydroxybutyrate (μmol/L)	31 ± 6	35 ± 12	65 ± 25	303 ± 125	<.01
Acetoacetate (μmol/L)†	72 ± 4	76 ± 16	102 ± 15	205 ± 64	.08
Lactate (mmol/L)	0.6 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	.26
Insulin (pmol/L)	191 ± 56	158 ± 34	41 ± 4	49 ± 5	.52
C-peptide (pmol/L)	1,467 ± 171	1,498 ± 274	368 ± 32	502 ± 59	.56

NOTE. Data are the mean ± SE.

*Refers to the area under the curve measurements during infusion of saline *v* Intralipid.

†Data on 7 subjects only.

tions decreased to basal levels during the infusion period. There was no significant difference in the basal concentrations of insulin ($P = .52$) or C-peptide ($P = .56$) during the infusion period (Fig 1 and Table 1).

Intravenous Glucose Tolerance Test

Plasma FFA and blood glycerol. The elevated concentration of FFAs and glycerol achieved during infusion with Intralipid plus heparin declined toward baseline during the glucose tolerance test (Fig 2).

Blood glucose. There was no difference in the area under the curve ($P = .72$) or the peak concentration achieved during the glucose tolerance test ($P = .61$) or at any of the individual time points (Table 3).

Blood ketones. The elevated concentration of both 3-hydroxybutyrate and acetoacetate during infusion of Intralipid

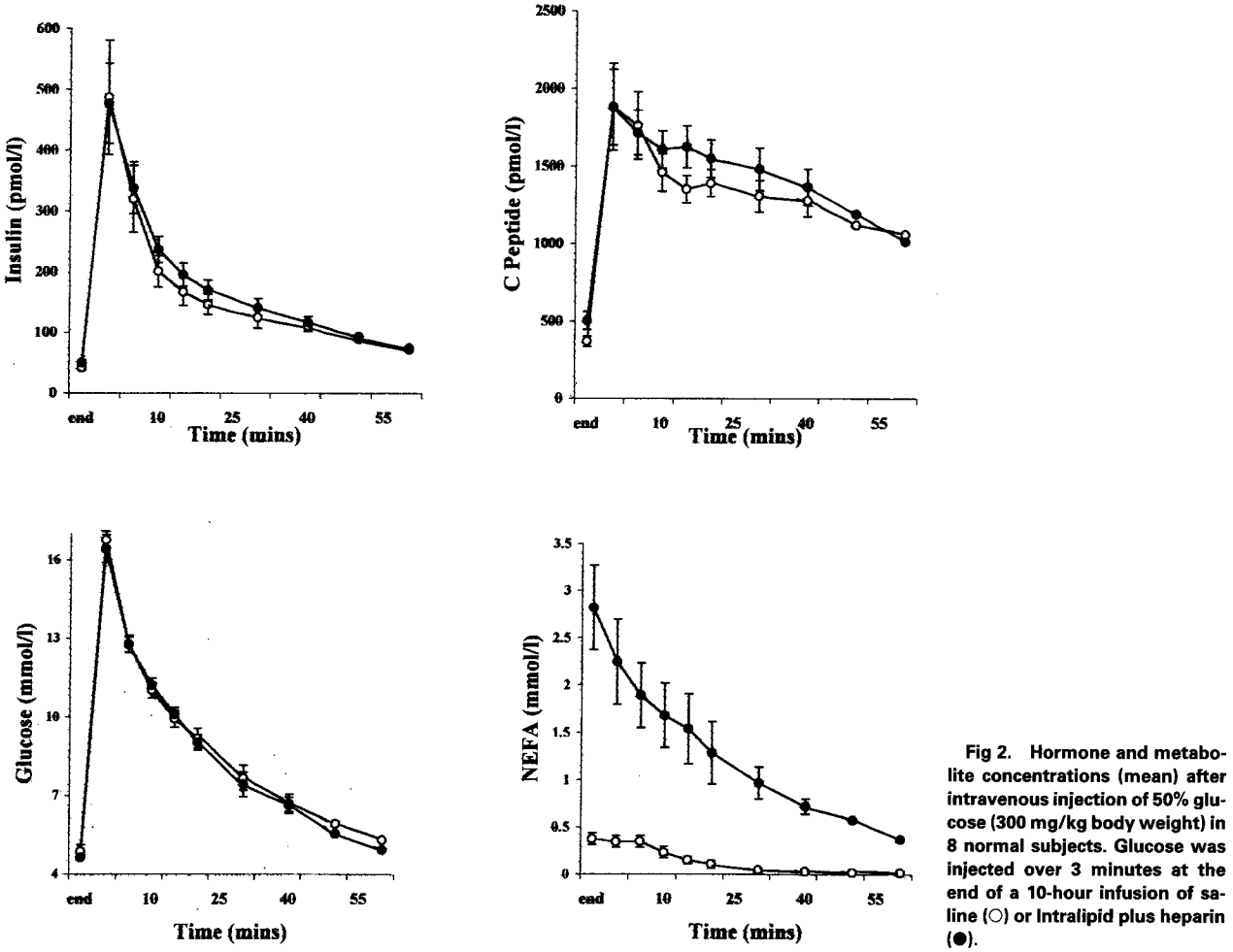

Fig 2. Hormone and metabolite concentrations (mean) after intravenous injection of 50% glucose (300 mg/kg body weight) in 8 normal subjects. Glucose was injected over 3 minutes at the end of a 10-hour infusion of saline (○) or Intralipid plus heparin (●).

Table 2. Peak Concentrations of Hormones and Glucose Achieved During Intravenous Glucose Tolerance Test After Infusion of Saline or Intralipid Plus Heparin in Eight Normal Subjects

Parameter	Saline	Intralipid	*P*
Glucose (mmol/L)	16.8 ± 0.3	16.4 ± 0.5	.61
Insulin (pmol/L)	489 ± 93	493 ± 60	.51
C-peptide (pmol/L)	2,025 ± 254	1,978 ± 216	.83

NOTE. Data are the means ± SE.

plus heparin decreased toward baseline during the glucose tolerance test (Fig 2 and Tables 2 and 3).

Blood lactate. Intravenous injection of glucose caused a steady increase in the lactate concentration (Table 3). There was no significant difference in this increase between the infusions ($P = .2$).

Plasma insulin and C-peptide. The glucose tolerance test provoked a peak increase in insulin and C-peptide which gradually declined during the 1-hour sampling period (Fig 2 and Tables 2 and 3). There was no statistical difference between the peak concentrations achieved for insulin ($P = .51$) or C-peptide ($P = .83$) and no difference in the area under the curve measurements for this period for insulin ($P = .27$) or C-peptide ($P = .44$).

DISCUSSION

In this study, we have examined the effect of overnight infusion of Intralipid plus heparin on basal and glucose-stimulated insulin secretion in normal subjects. During infusion of Intralipid plus heparin, mean plasma FFA concentrations increased by over 9-fold, to about 3 mmol/L. This elevation in FFA was accompanied by a significant increase in both blood glycerol and 3-hydroxybutyrate concentrations. Despite the elevation in plasma FFAs, no effect on basal insulin, that is, the insulin concentration during the infusion, or glucose-stimulated insulin secretion was observed.

The effect of FFAs on insulin secretion is unclear. Data from animal studies suggest that under appropriate conditions, exposure to increased FFA concentrations may lead to either increased or decreased insulin secretion.[10,13] Thus, Zhou and Grill,[10] using isolated rat islets, observed a time-dependent effect, showing that 3 hours' exposure to an elevated concentration of FFA produced an increase in glucose-stimulated insulin secretion, but after 48 hours' exposure, there was a decrease in insulin secretion. Similar results were obtained by the same group using isolated human islets.[11] In human studies, conflicting results have been obtained. Boden et al[14] infused healthy human subjects with Liposyn II (Abbott, North Chicago, IL) and heparin. During a 48-hour hyperglycemic clamp, FFA concentrations of 1.2 mmol/L were obtained and an increase in insulin secretion was observed. In contrast, Paolisso et al[15] found a stimulatory effect on insulin secretion at 6 hours but an inhibitory effect after a 24-hour infusion of Intralipid in healthy subjects.

Our results may differ from those of Boden et al[14] for three reasons. Firstly, we obtained considerably higher FFA concentrations (3.0 *v* 1.2 mmol/L). Conversely, it should be noted that the duration of exposure to increased FFAs is not a factor since increased insulin secretion was observed 4 hours into the clamp, which was the first time point examined. Secondly, we examined glucose-stimulated insulin secretion at basal glucose concentrations. Thus, at the start of the glucose tolerance test, the mean blood glucose in our normal subjects after Intralipid and heparin infusion was 4.6 mmol/L, whereas Boden et al[14] performed their hyperglycemic clamps at a glucose concentration of 8.8 mmol/L. Interestingly, Zhou and Grill[10] observed that preincubation of rat islets with a glucose concentration of 11 mmol/L rather than 5.5 mmol/L augmented the stimulatory effect of palmitate. Thirdly, a further difference between the study of Boden et al[14] and the present study lies in the effect on ketone bodies of the methodology. Thus, in our study, 3-hydroxybutyrate levels during Intralipid and heparin infusion were about 300 μmol/L, whereas 3-hydroxybutyrate during the hyperglycemic clamp declined from about 70 μmol/L to approximately 30 μmol/L.

Paolisso et al[15] examined normal subjects by performing sequential intravenous glucose tolerance tests immediately before the infusion and at 6 and 24 hours of the infusion. Infusion of Intralipid and heparin in their study resulted in an elevation of FFAs to about 1.2 mmol/L at 6 hours, and this was accompanied by a significant increase in both plasma glucose and insulin. After the 6-hour test, subjects were allowed to eat a "standardized diet." At 24 hours, plasma glucose had increased further but plasma insulin declined to preinfusion levels. Thus, the 24-hour study was performed in subjects who were not

Table 3. Hormone and Metabolite Concentrations Immediately Before Intravenous Injection of 50% Glucose and After 60 Minutes of the Intravenous Glucose Tolerance Test

	End Infusion		GTT 60 min		
Parameter	Saline	Intralipid	Saline	Intralipid	*P**
FFA (mmol/L)	0.37 ± 0.06	2.82 ± 0.45	0.02 ± 0.01	0.37 ± 0.07	<.005
Glycerol (mmol/L)	0.04 ± 0.005	0.13 ± 0.009	0.01 ± 0.001	0.02 ± 0.001	<.001
Glucose (mmol/L)	4.9 ± 0.2	4.6 ± 0.1	5.3 ± 0.4	4.9 ± 0.3	.72
Hydroxybutyrate (μmol/L)	65 ± 25	303 ± 125	5 ± 2	109 ± 91	<.001
Acetoacetate (μmol/L)†	102 ± 15	205 ± 64	55 ± 11	90 ± 57	.23
Lactate (mmol/L)	0.4 ± 0.08	0.5 ± 0.07	0.7 ± 0.06	0.9 ± 0.2	.2
Insulin (pmol/L)	41 ± 4	49 ± 5	72 ± 8	74 ± 9	.27
C-peptide (pmol/L)	368 ± 32	502 ± 59	1,057 ± 104	1,012 ± 115	.44

NOTE. Data are the mean ± SE.

Abbreviation: GTT, glucose tolerance test.

*Refers to the area under the curve measurements during GTT.

†Data on 7 subjects only.

fasted. The interpretation of their results is complicated since the intravenous glucose tolerance tests were performed at significantly different basal glucose concentrations. Furthermore, no information is provided about the concentration of ketone bodies, which would be expected to differ between the 6-hour test performed in the fasted state and the 24-hour test performed in the fed state.

It is possible that we have performed our intravenous glucose tolerance tests at a time when the short-term insulin stimulatory effect of FFAs is changing to a longer-term inhibitory effect. This seems unlikely for a number of reasons. Firstly, we observed no effect on glucose and insulin during the 10-hour infusion, in contrast to Boden et al.[14] Secondly, the pattern of response of glucose and insulin during the intravenous glucose tolerance test was nearly identical. Thirdly, our infusion period is not dissimilar to that of the early infusion period of Paolisso et al,[15] who observed increased insulin secretion after 6 hours of elevated FFAs.

In our study, FFA infusion was accompanied by an increase in both ketone bodies and glycerol. Ketone bodies have an effect on insulin secretion, although whether it is stimulatory or inhibitory is unclear. Islets incubated with 3-hydroxybutyrate show either an increase[7] or a decrease[11] in insulin secretion. In vivo studies in dogs,[16] rats,[17] and humans[18] show an increase in circulating insulin and C-peptide in response to infusion of 3-hydroxybutyrate or acetoacetate. It is possible that an effect of FFAs on glucose-stimulated insulin secretion may be modified by circulating levels of ketone bodies. Glycerol does not affect insulin secretion.[19]

Intralipid is a fat emulsion with a fatty acid composition similar to that of human blood, albeit with a lower proportion of palmitate. Previous studies in vitro on the effect of different fatty acids on insulin secretion have shown similar effects of palmitate, oleate, myristate, stearate, palmitoleate, linoleate, linolenate, and γ-linolenate, while arachidonate has different effects versus other fatty acids.[20] Arachidonate is not found at a high concentration in either human plasma or Intralipid.

In summary, our findings suggest that a significant elevation of plasma FFAs for 10 hours has no effect on basal insulin secretion or glucose-stimulated insulin secretion in normal subjects studied at basal glucose concentrations. Our results differ from those reported previously, which may reflect methodological differences. In particular, effects on insulin secretion may require a degree of hyperglycemia to be manifest, reiterating the importance of performing similar studies in type 2 diabetes.

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