Effect of Fatty Acids on Glucose Production and Utilization in Man

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ABSTRACT Since the initial proposal of the glucose fatty acid cycle, considerable controversy has arisen concerning its physiologic significance in vivo. In the present study, we examined the effect of acute, physiologic elevations of FFA concentrations on glucose production and uptake in normal subjects under three controlled experimental conditions. In group A, plasma insulin levels were raised and maintained at ~100 μU/ml above base line by an insulin infusion, while holding plasma glucose at the fasting level by a variable glucose infusion. In group B, plasma glucose concentration was raised by 125 mg/100 ml and plasma insulin was clamped at ~50 μU/ml by a combined infusion of somatostatin and insulin. In group C, plasma glucose was raised by 200 mg/100 ml above the fasting level, while insulin secretion was inhibited with somatostatin and peripheral glucagon levels were replaced with a glucagon infusion (1 ng/min·kg). Each protocol was repeated in the same subject in combination with a lipid-heparin infusion designed to raise plasma FFA levels by 1.5–2.0 μmol/ml.

With euglycemic hyperinsulinemia (study A), lipid infusion caused a significant inhibition of total glucose uptake (6.3±1.3 vs. 7.4±0.6 mg/min·kg, $P < 0.02$). Endogenous glucose production (estimated by the [3-^{3}H]glucose technique) was completely suppressed both with and without lipid infusion. With hyperglycemic hyperinsulinemia (study B), lipid infusion also induced a marked impairment in glucose utilization (6.2±1.1 vs. 9.8±1.9 mg/min·kg, $P < 0.05$); endogenous glucose production was again completely inhibited despite the increase in FFA concentrations. Under both conditions (A and B), the percentage inhibition of glucose uptake by FFA was positively correlated with the total rate of glucose uptake ($r = 0.69$, $P < 0.01$). In contrast, when hyperglycemia was associated with relative insulinopenia and hyperglucagonemia (study C), thus simulating a diabetic state, lipid infusion had no effect on glucose uptake (2.9±0.2 vs. 2.6±0.2 mg/min·kg) but markedly stimulated endogenous glucose production (1.4±0.5 vs. 0.5±0.4 mg/min·kg, $P < 0.005$). Under the same conditions as study C, a glycerol infusion producing plasma glycerol levels similar to those achieved with lipid-heparin, enhanced endogenous glucose production (1.5±0.5 vs. 0.7±0.6 mg/min·kg, $P < 0.05$).

We conclude that, in the well-insulinized state raised FFA levels effectively compete with glucose for uptake by peripheral tissues, regardless of the presence of hyperglycemia. When insulin is deficient, on the other hand, elevated rates of lipolysis may contribute to hyperglycemia not by competition for fuel utilization, but through an enhancement of endogenous glucose output.

INTRODUCTION

Almost 20 years ago, Randle and his associates (1) demonstrated an inhibitory effect of FFA on glucose metabolism in isolated rat hearts and hemidiaphragms. They showed that the addition of various fatty acids to the perfusion medium of these muscles leads to inhibition of glucose transport and phosphorylation, with accumulation of free intracellular glucose, and to inhibition of glycolysis and pyruvate oxidation. The mechanisms of these effects were clarified in studies by the same group (2, 3) showing that under aerobic conditions increased provision of FFA causes an increase in FFA oxidation, resulting in a buildup of mytochondrial citrate and acetyl-coenzyme A (CoA), and in a rise in the cytoplasmic NADH/NAD ratio. The increase in acetyl-CoA can inhibit pyruvate dehydrogenase activity and lead to augmented release

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of lactate. Citrate (a potent inhibitor of phosphofructokinase) slows down glycolytic flux and hence net glucose utilization. A glucose-fatty acid cycle was delineated (4), whereby in adipose tissue and muscle, glucose promotes reesterification of circulating FFA via enhanced formation of glycerolphosphate, whereas FFA restrict glucose utilization via the inhibition of glycolysis in muscle. Since metabolic changes similar to those produced by addition of FFA (or ketone bodies) to muscles of normal fed rats were also observed in perfused hearts and diaphragms of alloxan-diabetic rats, Randle and co-workers (4, 5) proposed that high plasma concentrations of FFA were responsible for the reduced insulin sensitivity of diabetes mellitus.

Subsequent studies investigating the operation of the glucose fatty acid cycle in skeletal muscle have yielded conflicting results. Thus, in rat diaphragms Schonfeld and Kipnis (6) could not reproduce the findings of Randle and co-workers (1–5). Likewise, in sartorius muscles of the rhesus monkey (7, 8), and in isolated perfused rat hemi-corpus (9) and hindquarters (10–12), the addition of fatty acids did not inhibit glucose metabolism. Contrary evidence, on the other hand, has been obtained in the exercising rat (13), in well oxygenated perfused rat skeletal muscle (14), and in the dog (15–17).

In man, Felber and Vannotti (18) first reported that fat infusion causes glucose intolerance in normal subjects. Nestel and co-workers (19) found that the rate of removal of an intravenous glucose load from plasma during norepinephrine infusion was accelerated if the norepinephrine-induced rise in plasma FFA was prevented by nicotinic acid. Similarly, Schalch and Kipnis (20) observed a slower fractional rate of plasma glucose disappearance when the intravenous glucose tolerance test was preceded by a fat meal-heparin regimen in normal but not in diabetic subjects. Finally, Balasse and Neef (21) reported a small (9%) decline in the rate of plasma glucose disappearance (measured with [^{14}C]glucose) after triglyceride infusion in normal subjects in the fasting state. Pelkonen et al. (22), however, reached the conclusion that FFA do not influence the tolerance to intravenous glucose administration in healthy man.

Another possible interaction of FFA with glucose metabolism is stimulation of hepatic glucose production. Although propionate, one of the end products of odd-chain fatty acid oxidation, can be converted in man into succinate and, ultimately, to glucose (23), human adipose tissue contains very little odd-chain fatty acids (24). Early studies have shown that FFA stimulate gluconeogenesis from lactate (25, 26), alanine (27), or pyruvate (28), in the perfused rat liver. In vitro, the mechanism for this effect is not an increased supply of carbons for glucose synthesis, but activation of the key enzymes of gluconeogenesis (29). Exton and his associates (30), however, found that in the perfused rat liver, albumin-bound oleate in physiologic concentrations fails to stimulate gluconeogenesis strongly. These authors have therefore questioned the physiological role of FFA in gluconeogenesis (31). In vivo proof of the putative influence of FFA on glucose formation is not available, to our knowledge.

In view of the potential importance of glucose fatty acid interactions in clinical states of impaired insulin sensitivity (4), we undertook to examine in man the consequences of physiological elevations of FFA concentrations on glucose production and utilization under controlled experimental conditions mimicking the normal fed and the diabetic state.

METHODS

Subjects

A total of 52 studies were carried out on 26 healthy volunteers (9 women and 17 men) ranging in age from 18 to 34 yr (mean±SEM = 24±1), and within 20% of their desirable body wt (103±2%, based on the Metropolitan Life Insurance Co. Tables, 1959). Their absolute body weights ranged from 48 to 90 kg (mean = 68±2 kg). All subjects were consuming a weight-maintaining diet containing at least 200 g carbohydrate/d for 3 d before study. No subjects were taking any medication. None had a family history of diabetes mellitus. The purpose, nature, and potential risks of the study were explained to all subjects, and written consent was obtained before their participation. The protocol was reviewed and approved by the Committee on Human Investigation at Yale University School of Medicine.

Experimental protocols

All subjects were studied in the postabsorptive state at 8 a.m. after a 12–14 h overnight fast. A polyethylene catheter was inserted into an antecubital vein for the infusion of all the test substances. Another catheter was inserted retrograde into a wrist vein for blood sampling. The hand was kept in a heated chamber at 70°C to allow arterialization of the venous blood (32).

Three protocols were used in three different groups of subjects.

Group A: hyperinsulinemia with euglycemia. In six subjects, peripheral plasma insulin concentration was acutely raised by ~100 μU/ml by a primed-continuous infusion of insulin (Eli Lilly and Co., Indianapolis, IN) while plasma glucose was maintained at fasting levels by the frequent adjustment of a variable glucose infusion (insulin clamp technique [32]).

Group B: hyperinsulinemia with hyperglycemia. In seven subjects, glucose was infused to acutely raise and maintain plasma glucose at 125 mg/100 ml above the fasting level with the use of the hyperglycemic clamp technique (32). 10 min before starting the glucose infusion, a constant infusion of cyclic somatostatin (SRIF,[1] 300 μg/h, kind gift of Drs. J.

[1] *Abbreviations used in this paper:* I, plasma insulin concentration; M, glucose uptake; SRIF, cyclic somatostatin.

Rivier and R. Guillemin, Salk Institute, San Diego, CA) insulin (0.3 mU/min · kg), and glucagon (0.65 ng/min · kg) was begun, and continued throughout the study.

Group C: hyperglycemia with basal insulin levels. In seven subjects, plasma glucose levels were gradually (over ~30 min) raised by, and maintained at, 200 mg/100 ml above the fasting level by the hyperglycemic clamp technique (32). 10 min before starting the glucose infusion, an infusion of SRIF (300 μg/h) was begun and continued throughout the study. In these experiments, however, insulin was not infused, and glucagon (Eli Lilly and Co.) was instead co-infused with SRIF, at the rate of 1 ng/min · kg. This protocol was designed to simulate the chief metabolic features (hyperglycemia, hypoinsulinemia, hyperglucagonemia) of the diabetic state.

With each of the three protocols, the subjects were restudied on a different day. In the paired studies, the infusion protocols were identical except that on one occasion an infusion of Intralipid (a 20% triglyceride emulsion, kindly provided by Cutter Laboratories, Berkeley, CA) was also given, at the rate of 1.5 ml/min, together with heparin (a bolus of 200 U plus 0.4 U/min · kg) to raise plasma FFA concentrations three- to fourfold. It has been previously shown that, under these circumstances, the increase in plasma FFA levels is due to intravascular lipolysis of the infused triglycerides under the influence of heparin, which stimulates the activity of lipoprotein lipase in blood (33). The order of the control and test (lipid infusion) studies was randomized. All studies lasted 2 h from the start of glucose/insulin infusion. When hyperglycemia was created, the subjects voided at the end of the study, and urine volume and glucose content were measured.

Under conditions of euglycemic or hyperglycemic hyperinsulinemia such as those created with protocols A and B, many studies have shown that endogenous glucose production is completely suppressed in the normal subject (34–39). In groups A and B in this study, therefore, endogenous glucose production was measured only in two subjects in each group, to rule out that lipid infusion might release the inhibition of glucose production induced by the raised insulin and/or glucose concentrations. When hyperglycemia is associated with low insulin levels, on the other hand, glucose output may be abated but not wholly abolished (35, 36, 39). Endogenous glucose production was therefore measured in all seven subjects in group C. Endogenous glucose production was quantitated by a primed-constant infusion of [3-^{3}H]glucose (New England Nuclear, Boston, MA) as previously described (40). The tracer infusion was begun 2 h before the glucose/insulin infusions, and continued throughout the experiment.

Six additional subjects were studied according to protocol C except that glycerol (a 2 M solution in water), instead of Intralipid, was infused at an average rate of 0.75 mmol/min to simulate the rise in plasma glycerol levels observed after lipid infusion. Tracer glucose was used in all the subjects in this group.

Analytical procedures

Plasma glucose was measured in duplicate by the glucose oxidase method on a Beckman Analyzer (Beckman Instruments, Inc., Fullerton, CA). Plasma [^{3}H]glucose radioactivity was measured as described (40). Plasma insulin and glucagon were measured by radioimmunoassay. Plasma FFA (41), glycerol (42), triglycerides (43), lactate (44), and β-hydroxybutyrate (45) were assayed by standard methods.

Data analysis

In protocols A and B, in which endogenous glucose output at the steady state was not significantly different from zero, total glucose uptake was calculated as the difference between the exogenous glucose infusion and urinary glucose loss. In protocol C and in the glycerol infusion experiments, total rates of glucose appearance and disappearance were calculated from the [^{3}H]glucose data (40). Total glucose uptake was then obtained as the difference between the rate of glucose disappearance from plasma and urine glucose excretion, while the rate of endogenous glucose production was calculated as the difference between the tracer-determined rate of total glucose appearance in plasma and the exogenous glucose infusion rate.

All glucose turnover data were averaged over the 2nd h of the study, when a steady state was most likely to exist.

The *t* test, unpaired or paired as appropriate, was used to compare mean values. Linear regression analysis was performed by standard techniques.

RESULTS

Group A. When plasma insulin concentrations were raised by 100 μU/ml while holding plasma glucose constant at the fasting level (Fig. 1), plasma FFA con-

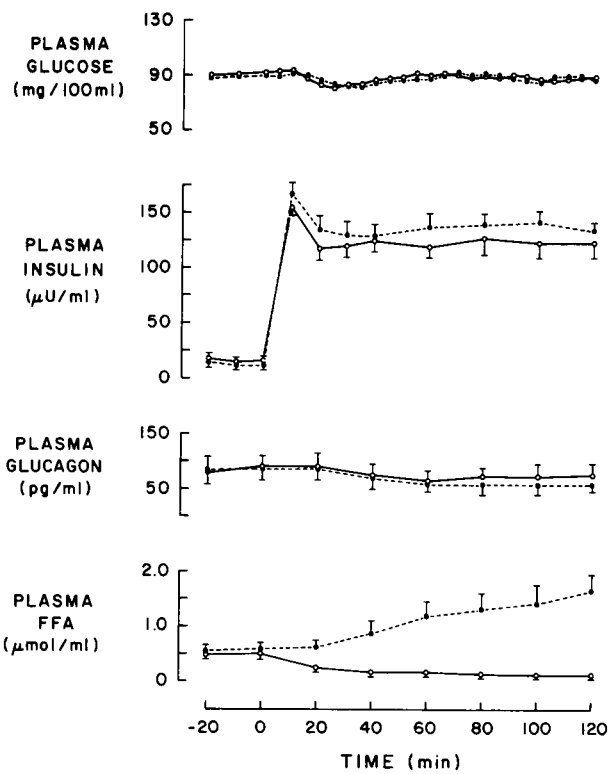

FIGURE 1 Time course of plasma glucose, insulin, glucagon, and FFA concentrations in group A subjects (n = 6) during the euglycemic insulin clamp (control study, shown by solid lines [○]) and during a euglycemic insulin clamp combined with intralipid-heparin infusion (dashed lines [●]). Vertical bars indicate 1 SEM.

TABLE I

*Plasma Glucose and Insulin Concentrations, and Rates of Glucose Uptake and Production in the Three Experimental Groups with and without Lipid Infusion**

	Group A (n = 6)		Group B (n = 7)		Group C (n = 7)	
	Control	Intralipid	Control	Intralipid	Control	Intralipid
Plasma glucose ($mg/100\ ml$)	89±3	89±3	204±4	206±4	291±3	287±3
Plasma insulin ($\mu U/ml$)	123±12	139±9	48±5	50±5	16±3	21±5
Glucose uptake ($mg/min \cdot kg$)	7.4±0.6	6.3±1.3§	9.8±1.9	6.2±1.1‡	2.6±0.2	2.9±0.2
Glucose production ($mg/min \cdot kg$)	−0.1	−0.4	−0.03	−0.3	0.5±0.4	1.4±0.5‖

* Average data of the 2nd h of the study.
‡ $P < 0.05$ vs. the respective control.
§ $P < 0.02$ vs. the respective control.
‖ $P < 0.005$ vs. the respective control.

centrations rapidly fell from a mean basal value of 0.48±0.10 μmol/ml to 0.15±0.03 during the 2nd h of the study ($P < 0.01$). During the lipid infusion study, very similar levels of plasma glucose and insulin were maintained, whereas plasma FFA concentrations gradually rose from 0.56±0.10 to 1.41±0.31 μmol/ml ($P < 0.01$). Under these conditions of euglycemic hyperinsulinemia, total glucose uptake was significantly lower (6.3±1.3 vs. 7.4±0.6 mg/min kg, $\Delta = 1.1 \pm 0.3$, $P < 0.02$) during lipid infusion than in the control study (Table I). When glucose uptake (M) was divided by the steady state plasma insulin concentration (I) to provide a measure of insulin-mediated glucose uptake, lipid infusion caused a 27±5% decrease in the M/I ratio ($P < 0.01$). Furthermore, the M/I ratio was inversely correlated with the logarithm of plasma FFA concentrations ($y = 90 - 6x$, $r = 0.60$, $P < 0.05$).

In the two subjects in whom it was measured, basal endogenous glucose production rates were completely suppressed both in the control clamp and during lipid infusion (Table I).

Plasma glucagon concentrations fell slightly both with and without lipid infusion (Fig. 1), whereas plasma triglycerides tended to decrease in the control study, and rose 2½-fold during lipid infusion (Fig. 2). Plasma glycerol concentrations remained at the base line in the control study, and rose three- to fourfold during Intralipid administration. Plasma β-OH-butyrate declined by 40% on the average ($P < 0.05$) during the 2nd h of the control study, whereas it failed to fall from the fasting value with lipid infusion ($P < 0.01$ vs. the control study, Fig. 2). Plasma lactate concentrations (not shown) were only modestly increased by euglycemic hyperinsulinemia in both studies.

Group B. When moderate hyperinsulinemia (50 μU/ml) was combined with hyperglycemia (200 mg/100 ml) (Fig. 3), plasma FFA levels fell from a fasting value of 0.55±0.08 μmol/ml to 0.15±0.03 between 60 and 120 min ($P < 0.01$), and total glucose uptake was greatly stimulated (9.8±1.9 mg/min·kg, Table I). When similar conditions of hyperglycemic hyperinsulinemia were associated with a threefold elevation in plasma FFA levels (Fig. 3), glucose uptake was markedly impaired as compared with the control study (6.2±1.1 mg/min·kg, $\Delta = 3.6 \pm 1.4$, $P < 0.05$, Table I). The M/I ratio was 38±10% lower ($P < 0.05$) during lipid infusion, and was inversely related to the logarithm of plasma FFA levels ($y = 36 - 3.3x$, $r = 0.54$, $P < 0.05$). In the two subjects in whom it was measured, glucose production was completely suppressed during

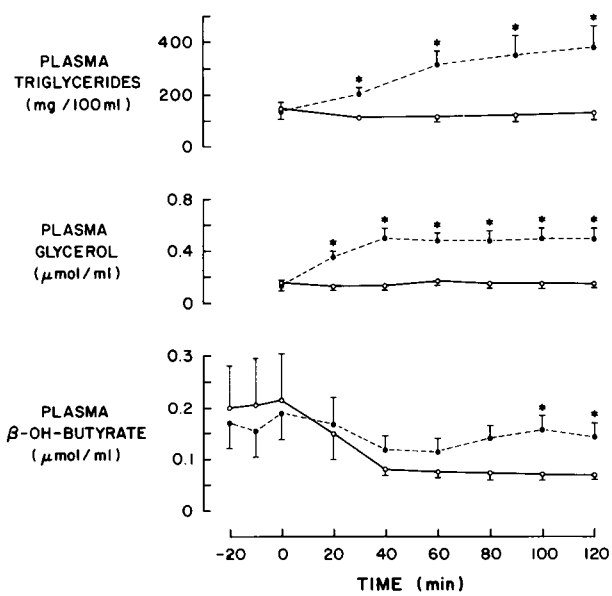

FIGURE 2 Time course of plasma triglycerides, glycerol, and β-OH-butyrate concentrations in group A subjects. Symbols are as in Fig. 1. *, $P < 0.05$ or less for the difference between mean values in the control and lipid infusion studies (paired t test).

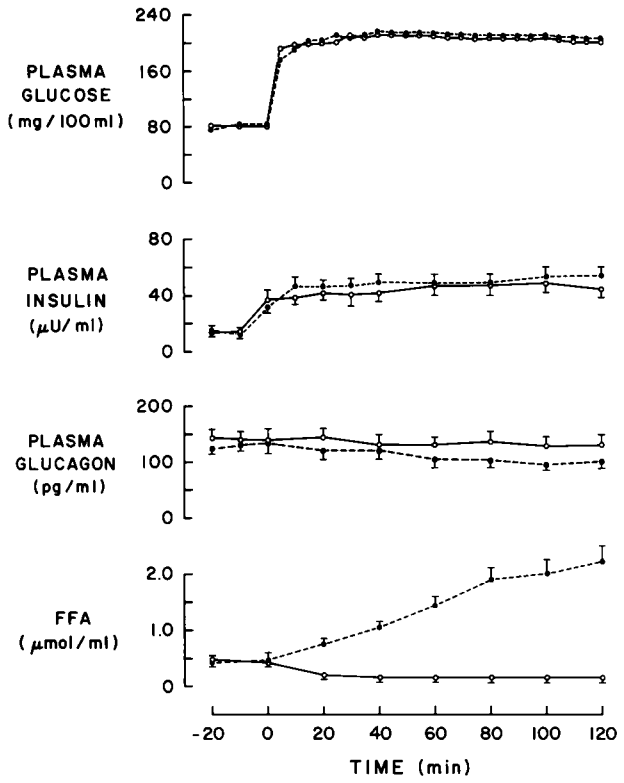

FIGURE 3 Time course of plasma glucose, insulin, glucagon, and FFA concentrations in group B subjects (n = 7). Symbols as in Fig. 1.

the 2nd h of both the control and the lipid infusion study (Table I). Glycosuria was very similar in the two sets of experiments (0.09±0.04 and 0.13±0.06 mg/min·kg, respectively).

The patterns of change in the plasma levels of triglycerides, glycerol, and β-OH-butyrate were very similar to those observed in group A (Fig. 4).

Group C. In the seven subjects in this group, marked hyperglycemia (~300 ml/100 ml) was associated with insulin levels (16±3 μU/ml) that were similar to the fasting values (14±2 μU/ml), while peripheral glucagon levels were slightly, though not significantly, higher than base line (128±27 vs. 101±20 pg/ml) as a result of the exogenous glucagon infusion (Fig. 5). Under these conditions, plasma FFA concentrations rose modestly at 40 and 60 min, and then returned to base line. Total glucose uptake increased only by 36±10% (from a basal value of 1.9±0.1 mg/min·kg to 2.6±0.2, $P < 0.01$, Table I), while a substantial part of plasma glucose was lost in the urine (0.98±0.1 mg/min·kg, or ~8 g in 2 h). With lipid infusion, plasma FFA concentrations rose above 2 μmol/ml, while plasma glucose, insulin, and glucagon levels were maintained at levels very close to those of the control

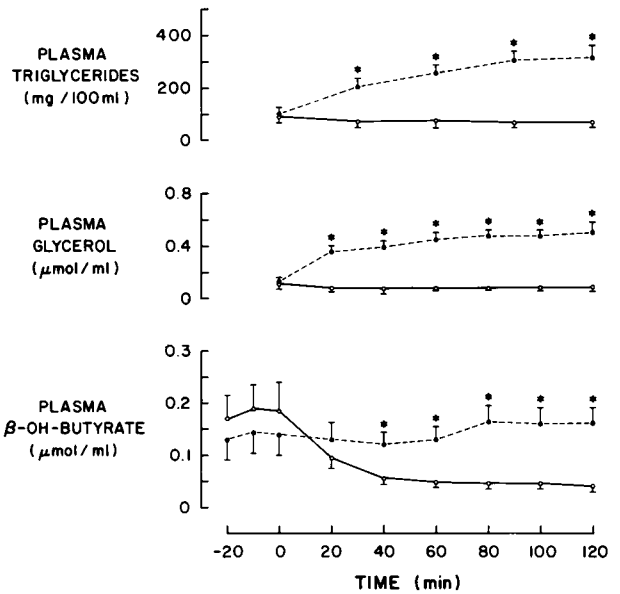

FIGURE 4 Time course of plasma triglycerides, glycerol, and β-OH-butyrate concentrations in group B subjects. Symbols as in Fig. 1.

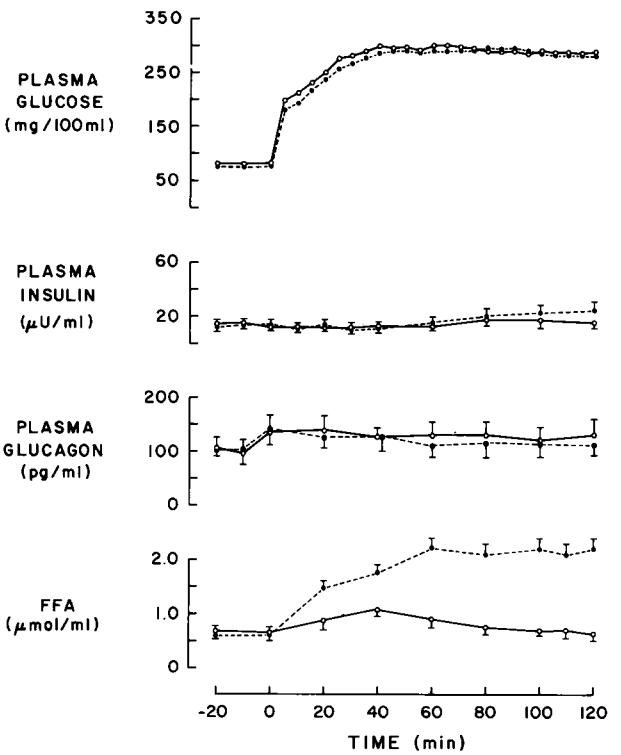

FIGURE 5 Time course of plasma glucose, insulin, glucagon, and FFA concentrations in group C subjects (n = 7). Symbols as in Fig. 1. Note that somatostatin infusion was begun at time −10 min (see Methods).

study. Under these conditions, basal glucose uptake, 1.8±0.1 mg/min·kg, was stimulated only by 60±13% ($P < 0.01$), to a mean rate, 2.9±0.2 mg/min·kg, that was not significantly different from that of the control study (Δ = 0.3±0.3 mg/min·kg, P = NS, Table I). Glycosuria, 1.0±0.1 mg/min·kg, was also very similar to that measured without lipid infusion.

Basal glucose output, 1.9±0.1 mg/min·kg, was suppressed by 82±22% ($P < 0.01$) in the control study. In contrast, during lipid infusion, endogenous glucose production diminished only slightly from base line (1.4±0.5 vs. 1.8±0.1 mg/min·kg, t = 0.97, P = NS) (Table I). Thus, lipid infusion caused a threefold enhancement in endogenous glucose production during the 2nd h of the study (Δ = 0.9±0.2 mg/min·kg, $P < 0.005$, Table I).

Plasma levels of triglycerides and glycerol showed a similar time course to those seen with the other two protocols (Fig. 6). β-OH-butyrate concentrations, however, were higher than basal during the 2nd h of the control study (0.40±0.07 vs. 0.27±0.06 μmol/ml, $P < 0.01$), and rose about three times after Intralipid administration (from 0.25±0.05 to 0.71±0.05 μmol/ml, $P < 0.001$).

When the data from groups A and B were pooled, a highly significant, direct correlation was found to exist between the degree of inhibition caused by FFA elevation and the individual rates of total glucose uptake (Fig. 7). The positive correlation persisted when the data from group C were included ($y = -22 + 5x$, $r = 0.72$, $P < 0.001$).

In six subjects, the study was performed as in group C except that glycerol, instead of Intralipid plus heparin, was infused for 2 h at an average rate, 0.75 mmol/min, designed to attain plasma levels similar to those seen after lipid/heparin infusion. The results (Table II) showed that glycerol infusion had no significant effect on glucose uptake but induced a definitive enhancement (+91±27%, $P < 0.05$) of endogenous glucose production (Fig. 8).

DISCUSSION

This study clearly demonstrates that acute, physiological elevations of plasma FFA levels inhibit insulin-stimulated glucose utilization, and that hyperglycemia does not overcome this inhibition. Since intravenously administered glucose in man is taken up chiefly by peripheral tissues (46), it is at this site that the observed effect of FFA must occur. The finding that the degree of inhibition of glucose uptake by FFA bore a direct relation to the absolute rate of glucose uptake (Fig. 7) also is compatible with the notion that muscle is the site of glucose and fatty substrate competition in normal man. The observed correlations between insulin-mediated glucose uptake and plasma FFA levels suggest that inhibition of lipolysis with the attendant fall in plasma FFA concentrations is one mechanism by which insulin promotes glucose utilization in vivo. By interpolating the present data, in fact, one can estimate that if FFA concentrations did not fall from base line during

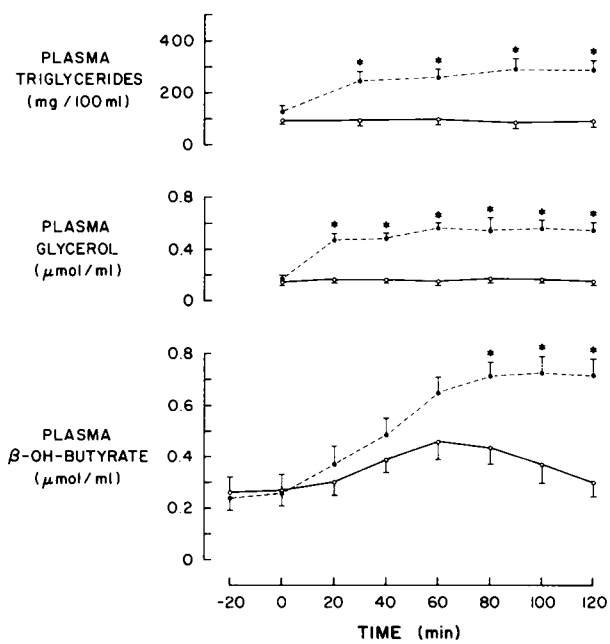

FIGURE 6 Time course of plasma triglycerides, glycerol, and β-OH-butyrate concentrations in group C subjects. Symbols as in Fig. 1.

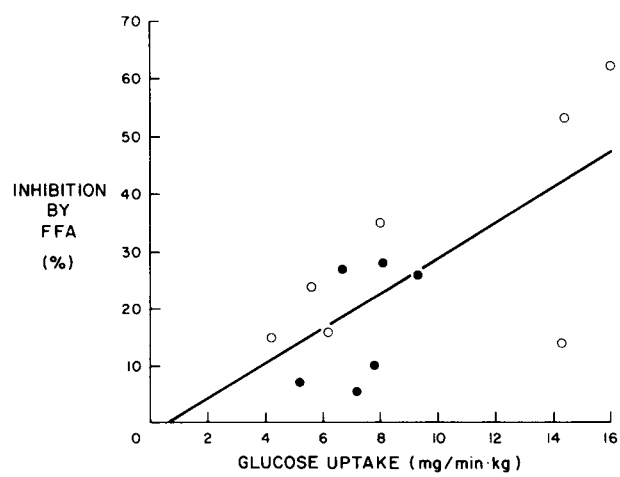

FIGURE 7 Relationship between percent inhibition of glucose uptake by Intralipid infusion and total rate of glucose uptake in 13 normal subjects under conditions of euglycemic (group A, ●) or hyperglycemic (group B, ○) hyperinsulinemia. $y = -1.7 + 3.1x$; $r = 0.69$; $P < 0.01$.

TABLE II

Biochemical Parameters in Six Subjects Receiving a Glycerol Infusion under the Conditions of Protocol C (See Methods)

	Control study			Glycerol infusion		
	Basal	Steady state	*P* (vs. basal)	Basal	Steady state	*P* (vs. basal)
Plasma glucose (*mg/100 ml*)	84±2	288±3	<0.001	85±3	293±3	<0.001
Plasma insulin (*μU/ml*)	9±1	7±1	NS	9±1	9±1	NS
Plasma glucagon (*pg/ml*)	148±29	178±34	<0.005	150±33	171±28	0.1 > *P* > 0.05
Plasma FFA (*μmol/ml*)	0.67±0.08	0.92±0.16	0.1 > *P* > 0.05	0.59±0.06	0.90±0.15	0.1 > *P* > 0.05
Plasma β-OH-butyrate (*μmol/ml*)	0.09±0.03	0.15±0.06	0.1 > *P* > 0.05	0.06±0.01	0.09±0.01	<0.05
Plasma glycerol (*μmol/ml*)	0.07±0.01	0.08±0.02	NS	0.07±0.01	0.41±0.06*	<0.001
Plasma triglycerides (*mg/100 ml*)	108±6	105±12	NS	117±18	167±13*	0.1 > *P* > 0.05

* $P < 0.05$ or less as compared with the control study.

a glucose-insulin clamp, glucose uptake would be 15–20% less.

In contrast to the insulinized state, when insulin levels were low and glucose flux was only modestly increased (group C), lipid infusion had no effect on glucose utilization. An explanation for this observation is that, in the absence of an insulin response to hyperglycemia, a great part of plasma glucose is taken up by insulin-independent tissues. In the latter (chiefly brain cells and erythrocytes), FFA do not interfere with glucose uptake, whereas the major site of competitive FFA and glucose utilization, the muscle, contributes little to overall glucose disposal because of the low insulin levels. It should also be noted that FFA (and ketone) levels failed to fall below base line in group C subjects even in the control experiments (i.e., without lipid infusion, Fig. 5) as opposed to the situation where hyperglycemia was accompanied by hyperinsulinemia (Fig. 3). One could therefore argue that inhibition of glucose uptake was present already in the control study (where lipolysis continued unabated), and that further elevation of FFA levels after lipid infusion produced no additional inhibition of glucose utilization.

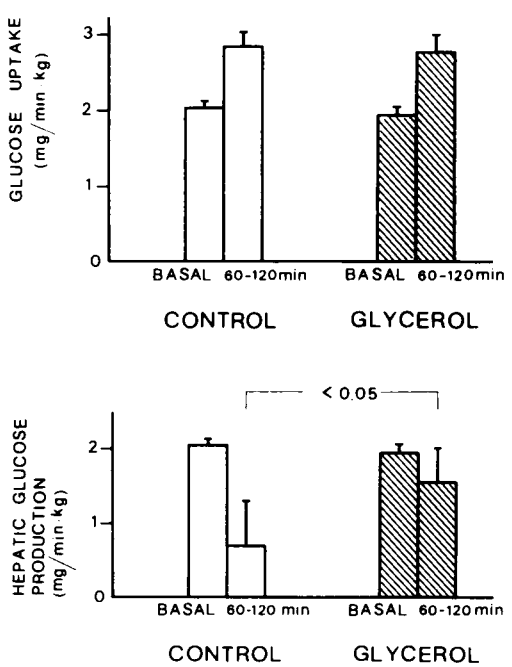

FIGURE 8 Glycerol infusion. Glucose uptake and production in six subjects receiving an infusion of glycerol under the experimental conditions of group C (see Methods and Table II).

Thus, to the extent that results from short-term experiments can be extrapolated to chronic metabolic states, it would appear that raised concentrations of FFA can induce glucose intolerance only if they persist in the face of an insulin response that directs high glucose fluxes into sensitive tissues, i.e., muscle. For example, high catecholamine output or excessive growth hormone secretion are conditions in which FFA can conceivably induce resistance to insulin action on glucose uptake, because of the concomitance of stimulated lipolysis and an effective insulin response (19, 20). In a like manner, when diabetic ketoacidosis is treated with insulin, the long-standing elevations in

FFA levels may oppose the blood-glucose lowering effect of insulin. Physical exercise is another situation in which FFA have been shown to competitively inhibit glucose uptake by muscle (13). In this case, glucose is driven into muscle at high rates in the presence of basal or decreased plasma insulin levels. Nevertheless, the marked acceleration of lipolysis brings forth an effective substrate competition, which conserves plasma glucose and spares muscle and liver glycogen (13). A fat meal ingested before exercise has been shown to amplify these effects (47). These observations suggest that it is not insulin per se, but a high rate of cellular glucose metabolism in insulin-dependent tissues that is necessary for FFA to exhibit their inhibitory effect on glucose uptake. In this regard, it is noteworthy that Schalch and Kipnis (20) were unable to reproduce in diabetic subjects the impairment in plasma glucose removal observed in normal subjects following a fat meal-heparin regimen.

On the other hand, if there is no insulin available to activate glucose transport into sensitive tissues, raised FFA levels are not likely to interfere with glucose utilization to any quantitatively significant degree. This conclusion agrees with the results of Paul et al. (48) who showed that pancreatectomized dogs given nicotinic acid to lower plasma FFA levels do not increase their rates of glucose oxidation. The clinical implication of this conclusion is that inhibition of peripheral glucose metabolism by FFA may have a relevant part in the insulin resistance of obesity and, possibly, type 2 diabetes with preserved pancreatic function (49), but plays a minor role in states of insulin deficiency such as untreated type 1 diabetes.

With regard to the effects of elevated FFA on the liver, the in vitro information is ambivalent (25–28, 31). In vivo, a stimulation of gluconeogenesis has been difficult to demonstrate because of the concomitant changes in the hormonal milieu. Thus, in dogs with portacaval shunts, acutely raising FFA levels produced a considerable increase in insulin secretion and a decrease in glucagon release, which concurred to lower hepatic glucose output (16). Conversely, in normal man nicotinic acid administration causes a fall in plasma FFA concentrations but a rise in hepatic glucose output mediated by a decrease in plasma insulin and an increase in plasma glucagon levels (50). In general, FFA are insulin secretagogues (51, 52), and their effect on plasma insulin levels counteracts any hyperglycemic action in vivo. In pancreatectomized dogs, however, reduction of circulating FFA by sodium nicotinate brings about a significant decrease in hepatic glucose production (48). Although indirect, the latter is the only in vivo evidence for a hepatic action of FFA on glucose release. In the present studies, lipid plus heparin infusion markedly enhanced glucose production during insulinopenia despite the presence of hyperglycemia, a factor which restrains hepatic glucose release (34–39). The control experiments showed that this effect could be produced also by increasing the supply of the gluconeogenic substrate, glycerol. Whether or not the rise in glucose output observed during lipid plus heparin infusion was solely due to a substrate effect cannot be determined with certainty. Some calculations, however, are worth carrying out. In 3-d-fasted, anesthetized dogs, glycerol levels similar to those achieved in the present experiments cause a net hepatic glycerol uptake of 6–8 μmol/min·kg (53). If quantitatively converted, this could account for 3–4 μmol/min·kg (or 0.5–0.7 mg/min·kg) of newly synthesized glucose. In postabsorptive man, splanchnic glycerol clearance is in the range of 11–13 ml/min·kg (54), which, at circulating glycerol levels of 0.4–0.6 μmol/ml (Fig. 6 and Table II), would contribute 0.4–0.7 mg/min·kg of glucose to gluconeogenesis. In the present study, the mean difference in endogenous glucose production was 0.75±0.29 mg/min·kg with glycerol infusion, whereas it was 0.90±0.18 mg/min·kg ($0.2 > P > 0.1$) with lipid plus heparin. Thus, a substrate effect could explain 50–90% of the increase in glucose output caused by glycerol alone, and 40–75% of that induced by lipid plus heparin. With all the above approximations, it can be concluded that elevated FFA concentrations may have an effect of their own on glucogenesis during insulinopenia; however, this effect is likely to be small. Furthermore, many other factors, such as the presence of hyperglycemia, the duration of exposure to high FFA/low insulin levels, and the metabolic state of the subjects can conceivably interfere (31, 55–60).

Nevertheless, the pathophysiological relevance of the results of the present study is that during insulin deficiency increased lipolysis may be an important factor in glucose overproduction. Thus, in untreated type 1 diabetes or starvation, activation of gluconeogenesis by products of FFA oxidation (29) may conspire with increased supply of glucose precursors and stimulation of counterregulatory hormones to augment glucose production.

Concerning the mechanism of these interactions of FFA in glucose homeostasis, tissue uptake and oxidation of FFA are requisites for FFA-induced inhibition of glucose metabolism in muscle (1–3). In keeping with this notion, β-hydroxybutyrate levels in groups A and B in the present study failed to fall in response to insulin (61) (and were markedly raised in group C) when FFA turnover was accelerated by lipid infusion (Figs. 2, 4, and 6), suggesting an increased generation of ketone bodies from FFA and, therefore, an increased oxidation of FFA. In heart and skeletal muscle, FFA (and ketones) inhibit glycolysis, pyruvate oxi-

dation, and glucose transport (1–3, 10–14); glycogen is increased or spared. Under conditions of euglycemic or hyperglycemic hyperinsulinemia similar to those created in group A and B in the present study, glucose oxidation accounts for ~40% of total glucose uptake (46, 62). The fate of the remaining glucose disappearing from the circulation is not known with certainty. Glycolysis, glycogen synthesis, lipogenesis, and the hexose monophosphate shunt all are possible routes of intracellular glucose disposal. The precise quantitative impact of increased respiration of fat on each of these metabolic pathways may be very difficult to evaluate. Muscle glycogen content, some estimate of glycolytic flux, changes in the concentration of citrate, and other intermediates of glucose metabolism would all be relevant information for a detailed description of FFA/glucose interrelationship in human peripheral tissues (see reference 63 for an extensive review). It is also pertinent to note that different tissues may respond differently to changes in substrate supply. For instance, in red muscle fibers, which have a high oxidative capacity, glycolysis is restrained by the addition of lipid substrates to a greater extent than in white muscle (63).

While further work is clearly needed to define the mechanisms, the results of the present study are altogether compatible with the operation of the glucose fatty acid cycle in vivo: Glucose lowers plasma FFA levels (through insulin), and FFA effectively compete with glucose for uptake by tissues, mostly skeletal and myocardial muscle, under augmented metabolic demand. However, the concept that such substrate competition is responsible for the insulin resistance of diabetes mellitus (4) appears to hold true only to a limited extent. In type 2 diabetes, chronically raised FFA levels (64) may impair or delay glucose disposal in response to insulin. On the other hand, when insulin secretion is deficient (as in type 1 diabetes), increased provision of FFA is unlikely to influence an already depressed muscle glucose metabolism. Instead, stimulation of gluconeogenesis by accelerated lipolysis may make an important contribution to the hyperglycemia of insulin-deficient states in man.

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