The Multiphasic Profile of Free Fatty Acids During the Intravenous Glucose Tolerance Test Is Unresponsive to Exogenous Insulin

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As small increments in insulin concentration profoundly affect lipolysis, our goal was to describe the free fatty acid (FFA) profile during the frequently sampled intravenous glucose tolerance test (FSIGT) and determine if both endogenous and exogenous insulin influenced the FFA profile. Thirteen subjects had both a glucose-only (GO-FSIGT) and insulin-modified FSIGT (IM-FSIGT). Both protocols were of 6 hours duration. At baseline an intravenous glucose bolus (0.3 g/kg) was given. In the IM-FSIGT, insulin was infused from 20 to 25 minutes (4 mU/kg · min). Six additional subjects had both an IM-FSIGT and a normal saline study (NS-Study). For the NS-Study, normal saline solution was infused instead of glucose and insulin. Fasting glucose, insulin, FFA and epinephrine concentrations were similar for all tests. Endogenous insulin peaked at 4 ± 1 minute in both FSIGTs. The mean calculated peak time of exogenous insulin in the IM-FSIGT was 26 ± 1 minute. Glucose concentrations were lower and epinephrine concentrations higher in the IM-FSIGT versus GO-FSIGT. During the FSIGTs, the FFA time course revealed four distinct phases, which did not differ between protocols. In phase I (0 to 11 minutes), FFA levels remained near basal (491 ± 183 μmol/L); in phase II (11 to 79 minutes), FFA levels declined achieving a nadir of 139 ± 63 μmol/L; in phase III (79 to 188 minutes), FFA levels rose linearly and reattained basal levels; and in phase IV (188 to 360 minutes), FFA levels rose above basal and plateaued at 732 ± 214 μmol/L ($P < .001$). In the NS-Study, FFA levels remained near baseline (388 ± 118 mEq/L) until 180 minutes and then trended upward to 618 ± 258 μmol/L at 360 minutes. FFA concentrations from 180 to 360 minutes did not differ in the IM-FSIGT versus NS-Study. As the 4 FFA phases did not differ between protocols, the insulin effect on FFA levels in the FSIGT can be attributed to endogenous insulin. But the similarity in FFA levels from 180 to 360 minutes in the IM-FSIGT and NS-Study suggests diurnal variation and not a dynamic related to insulin or the FSIGT protocol is responsible for the final suprabasal FFA plateau.

DEFECTS IN insulin's ability to regulate free fatty acids (FFA) contribute to the development of insulin resistance and type 2 diabetes.[1,2] Clinically useful tools to assess insulin's ability to regulate glucose have been available for more than 30 years,[3,4] but there is no clinically practical method for the quantification of insulin's action on FFA.

During the hyperinsulinemic euglycemic clamp, FFA are totally suppressed at the standard insulin dose of 40mU · min^{-1} · m^{-2}.[5] Therefore, the hyperinsulinemic euglycemic clamp does not allow segregation of subjects based on FFA sensitivity to insulin. Hence, stepwise low-dose insulin clamp studies were designed and demonstrated that plasma FFA decline dramatically in response to very small increases in insulin concentration.[6] However, the low-dose insulin clamp is technically difficult to perform because several doses of insulin are required to define the ED_{50} for FFA suppression.

Subsequent to the euglycemic clamp, mathematical modeling of frequently sampled intravenous glucose tolerance test (FSIGT) data using the minimal model approach was developed, yielding the insulin sensitivity index (S_I).[3] Unlike the clamp, the FSIGT is a dynamic test in which glucose is injected as a single bolus at baseline and the subsequent time course of glucose and insulin are examined. After the introduction of the minimal model, it was determined that adding a bolus of insulin at 20 minutes to the FSIGT protocol led to improved resolution of S_I.[7,8] This work with the FSIGT did not include assessment of FFA. As insulin concentration changes over a wide range during the FSIGT, this method may provide insight into insulin-FFA interaction. The value of exploring the use of the insulin-modified FSIGT (IM-FSIGT) protocol in this context is that a single test could then be used to describe the relationship between insulin and glucose and insulin and FFA.

Two steps are required to assess if the IM-FSIGT is a useful tool to isolate the FFA-insulin relationship. First, the features of the FFA profile during the FSIGT have to be described and the susceptibility of the FFA profile to changes by both endogenous. Second, exogenous insulin needs to be determined. The objective of this study was to address these 2 issues.

MATERIALS AND METHODS

Nineteen healthy African-Americans (13 men and 6 women; mean age ± SD, 36 ± 6 years; range, 23 to 42 years; body mass index [BMI], 31.4 ± 8.3 kg/m^2; range, 22.3 to 56.9; S_I, 4.17 ± 2.82 L/mU · min^{-1}; range, 0.3 to 9.93; acute insulin response to glucose [AIRg], 678 ± 450 mU/L · min; range, 24 to 2,164) were enrolled in a protocol to evaluate

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insulin resistance. Three subjects were normal weight, 8 were overweight, and 8 were obese.[9] Recruitment was achieved using flyers, newsletters, and the National Institutes of Health (NIH) website. Subjects took no medications. All women were premenopausal and studied in the follicular phase of their cycle. The Institutional Review Board of NIH approved the study. Subjects gave informed consent.

Outpatient Visits

During visit 1, a medical history was taken and physical examination performed.

Visits 2 and 3 were performed in the morning after a 12-hour fast. The start time for the studies was between 7 and 8 AM. For each individual the start time of both studies was within 15 minutes.

During visit 2, an IM-FSIGT was performed. Intravenous catheters were placed in each antecubital vein. The arm used for phlebotomy was wrapped in a heating blanket. At time 0, glucose (0.3 g/kg) was injected over 1 minute and insulin (4 mU/kg · min) infused from 20 to 25 minutes. Samples for glucose, insulin, and FFA were obtained at −10, −1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes. Eight subjects had epinephrine levels determined at 0, 5, 10, 25, 60, 90, 120, 150, 180, 240, 300, and 360 minutes. Glucose was measured at the bedside using the glucose oxidase method (Glucostat; Yellow Springs Instruments, Yellow Springs, OH). Blood for insulin was drawn into serum separator tubes. Blood for FFA and epinephrine were drawn into chilled EDTA tubes, centrifuged immediately, and the plasma stored at −70°C. FFA assays were performed within 72 hours.

During visit 3, which was 4 weeks after visit 2, 13 consecutive subjects had a glucose-only FSIGT (GO-FSIGT) performed while 6 consecutive subjects had a normal saline control study (NS-Study). The GO-FSIGT was identical to the IM-FSIGT except that a normal saline solution infusion replaced the insulin infusion. For the NS-Study, normal saline solution was infused instead of glucose and insulin.

Analytic Methods

Insulin was measured with double-antibody chemiluminescent sandwich assays (Diagnostic Products, Los Angeles, CA). The cross-reactivity between insulin and proinsulin in this assay is 8%. FFA concentrations were assayed with enzymatic colorimetric kits (Wako Chemicals USA, Richmond, VA). Glucose, insulin, and FFA concentrations were determined in duplicate. The respective coefficients of variation were 1.9%, 3.2%, and 4.9%. Epinephrine concentrations were determined by liquid chromatography with electrochemical detection.[10]

Insulin sensitivity was determined as S_I from Minmod 3.[11] The AIRg was calculated as the integrated plasma insulin concentration above basal from 0 to 10 minutes.

Glucose Dynamics During the FSIGT

To determine if the rate of decline in glucose was different before and after the administration of insulin in the IM-FSIGT, regression analyses were performed with the log of glucose from 10 to 20 minutes and 20 to 60 minutes as dependent variables. Two independent variables were entered into the regression analyses: time and a dichotomous variable created to represent before and after 20 minutes. To maximally isolate the effect of insulin on glucose, the rate of glucose disappearance was calculated starting at 10 minutes. Prior to 10 minutes changes in glucose concentration are a function of extracellular mixing, glucose-mediated glucose disposal and insulin-mediated disposal.[12]

FFA Profile During the FSIGT

Observation of FFA concentrations against time revealed that the FFA profile during the FSIGT had 4 phases. These four phases are fully described in the Results section that follows. The mathematical calculations that are used to describe these results are provided here. Phase I was characterized by constancy of FFA near basal for several minutes following glucose injection. To objectively locate the extent of phase I, a modified logistic function was fitted. The end of phase I was located at the time of the minimum of the second derivative of the fit of this function. Phase II was defined as the interval between the beginning of the precipitous fall in FFA levels and the FFA nadir. The rate of decline of FFA in phase II was calculated as the slope of the line determined by log transformed FFA from 10 to 40 minutes. It is reported as the fractional disposal rate of FFA. The units are $\% \cdot min^{-1}$. They are calculated as the decline in FFA concentration, which occurred during that minute divided by the total FFA concentration at that minute multiplied by 100.

After the FFA nadir occurred, FFA ascended again. Phase III was defined as the period between the FFA nadir and the time at which FFA reattained basal levels. The rate of rise in FFA during this period was linear and calculated as the slope of the line from the FFA nadir to the time point when basal FFA levels were reattained. Phase IV was the time interval between the point when FFA reattained basal levels to the end of the study (360 minutes). In all subjects, by the end of phase IV, FFA levels achieved a suprabasal plateau.

Statistical Analyses

Data presented as means ± SD. Paired *t* tests were used to compare the 13 subjects who had both GO-FSIGT and IM-FSIGTs, and the 6 subjects who had both NS-Study and IM-FSIGTs. *P* values ≤ .05 were considered significant. Statistical analyses were performed with STATA 7 (College Station, TX).

RESULTS

Eighteen subjects had normal fasting glucose levels and 1 subject had impaired fasting glucose.[13] For the 13 subjects who had both IM-FSIGT and GO-FSIGT, fasting glucose, insulin, FFA, BMI, S_I, and AIRg were nearly identical (all $P \geq .40$). For the 6 subjects who had both IM-FSIGT and NS-Study, fasting glucose, insulin, FFA, and BMI were similar (all $P \geq .4$).

FSIGTs

From 0 to 19 minutes, the glucose and insulin patterns were not different in the GO-FSIGT and IM-FSIGT protocols (Figs 1 and 2). The infusion of insulin in the IM-FSIGT generated a second insulin peak, which was calculated to occur at 26 ± 1 minute (Fig 1A). Because of the insulin infusion, glucose concentrations were lower in the IM-FSIGT from 40 to 100 minutes (all $P \leq .01$) (Fig 2B). Thus, there was a prolonged, highly significant effect of exogenous insulin on glucose dynamics.

FFA Profiles

The FFA profile in the GO-FSIGT and IM-FSIGT had 4 phases. The FFA concentration during the GO-FSIGT and IM-FSIGT were not significantly different (Fig 2C and Table 1).

Phase I (0 to 11 minutes). FFA concentration changed minimally between 0 and 11 minutes. This "refractory" period of phase I ended when the abrupt decline in FFA concentration commenced. The duration of phase I was similar in the GO-FSIGT and IM-FSIGT (10 ± 7 *v* 11 ± 10 minutes, $P = .51$).

Phase II (11 to 79 minutes). This phase was characterized by plummeting FFA concentrations. Its end was identified with the nadir in FFA levels at 79 minutes. The decline in FFA levels during this phase is consistent with suppression of lipolysis. The importance of the role of endogenous insulin in

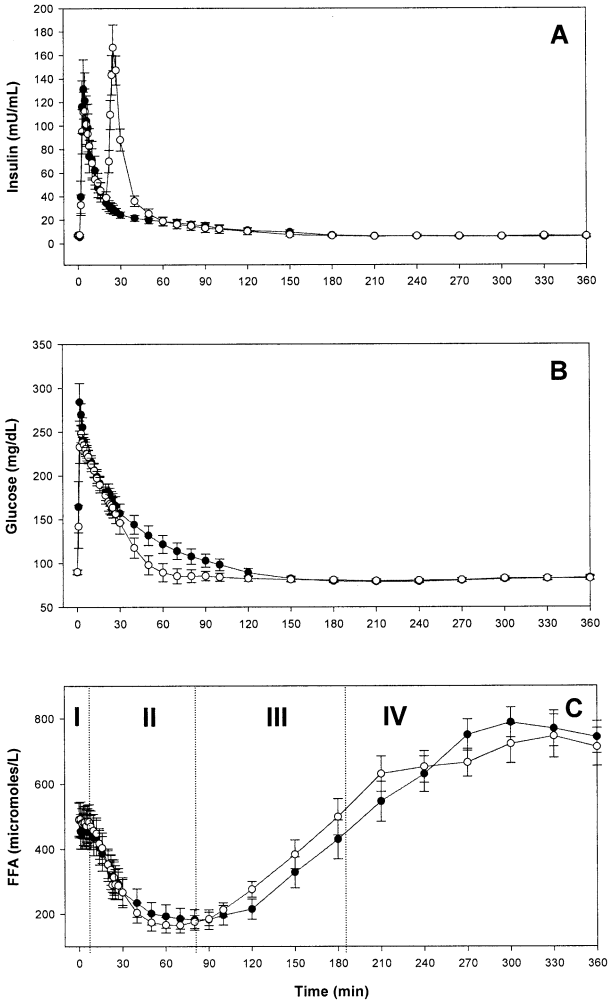

Fig 1. Time course of insulin, glucose, and FFA during the glucose-only (●) and insulin-modified (○) FSIGTs. (A) Insulin *v* time. (B) Glucose *v* time. (C) FFA *v* time; vertical bars demarcate the time points at which each FFA phase ends. Data presented as mean ± SE.

suppressing lipolysis is demonstrated by the fact that this second phase began *after* endogenous insulin levels peaked (4 ± 1 minutes) but *before* exogenous insulin was administered (20 minutes). Further, in the IM-FSIGT and GO-FSIGT, the maximum rate of decline of FFA (10% ± 4% *v* 11% ± 6% · min^{-1}, $P = .34$), the percent suppression of FFA (70.6% ± 10.6% *v* 71.3% ± 9.0%, $P = .80$) and the time of the FFA nadir (84 ± 30 *v* 79 ± 27 minutes, $P = .45$) were not different.

Exogenous insulin was administered in the IM-FSIGT at 20 minutes. To determine the effect of this exogenous insulin on FFA levels, the rate of decline of FFA between 10 and 20 minutes was compared to the rate of decline from 20 to 40 minutes. The rate of decline of FFA in the IM-FSIGT was not different in these intervals (2.6% ± 1.7% *v* 2.5% ± 1.8% · min^{-1}, $P = .79$). Furthermore, the maximum rate of decline of FFA in the IM-FSIGT occurred at 19.5 ± 5.4 minutes, which was before exogenous insulin was administered. Therefore, exogenous insulin had no measurable effect on the pattern or degree of decline of FFA.

Phase III (79 to 188 minutes). Phase III was defined as the time interval between the FFA nadir and when FFA levels reattained baseline values. In the 2 protocols, the rate of rise of FFA in phase III (5.11 ± 2.99 *v* 4.14 ± 2.15 $\mu mol \cdot L^{-1} \cdot min^{-1}$, $P = .27$) and the time FFA reattained basal (197 ± 48 *v* 188 ± 57 minutes, $P = .51$) were not different.

Phase IV (188 to 360 minutes). FFA continued to increase after 188 minutes. The moment at which the plateau began within phase IV was highly variable and difficult to precisely define because the sampling frequency for the last 3 hours of the study was every 30 minutes. However, in all subjects, by 360 minutes, FFA levels plateaued at a supra-basal level, which

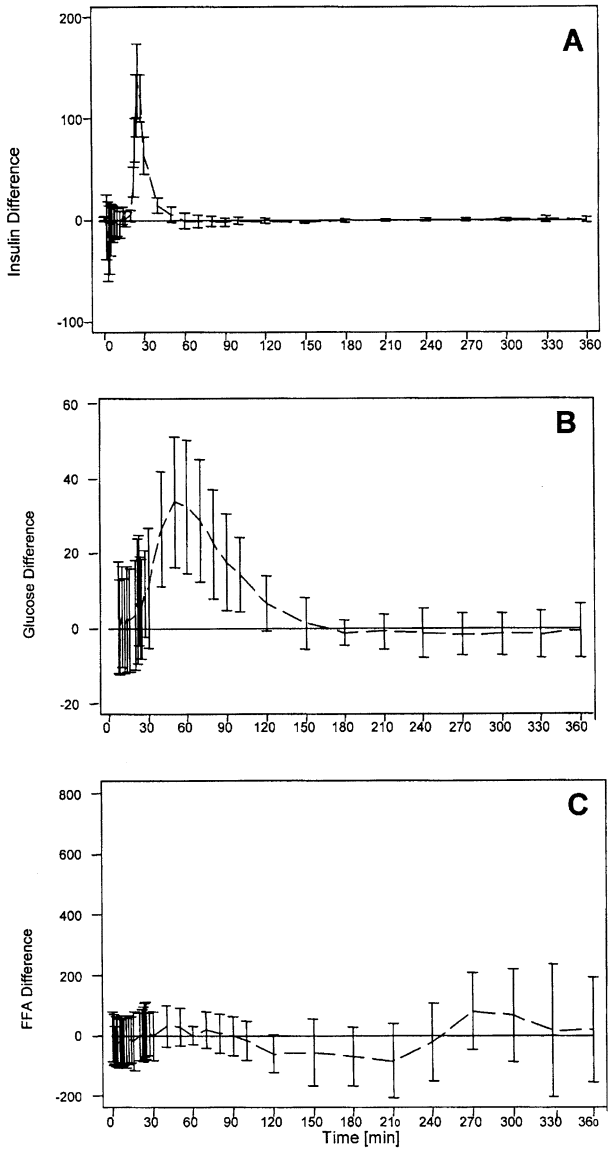

Fig 2. Time course of concentration differences of insulin, glucose, and FFA during the glucose-only *v* insulin-modified FSIGTs. (A) Insulin difference *v* time. (B) Glucose difference (multiplied by −1) *v* time. (C) FFA difference *v* time. Data presented as means and 95% confidence intervals.

Table 1. Features of the FFA Profile During the FSIGT

	GO-FISGT	IM-FISGT	*P* Value
Fasting FFA (μmol/L)	490 ± 194	491 ± 183	.96
Time phase I ended and phase II began (min)	10 ± 7	11 ± 10	.51
Time of maximum decrease in FFA during phase II (min)	21.9 ± 4.2	19.5 ± 5.4	.26
Maximum rate of decrease of FFA during phase II (μmol · L^{-1} · min^{-1})	30.2 ± 15	37.7 ± 21.3	.14
FFA concentration at nadir (μmol/L)	148 ± 97	139 ± 63	.70
Percent suppression of FFA during phase II	70.6 ± 10.6	71.3 ± 9.0	.80
Time phase II ended and phase III began (min)	84 ± 30	79 ± 27	.45
Rate of FFA rise in phase III (mEq · L^{-1} · min^{-1})	5.11 ± 2.99	4.14 ± 2.15	.27
Time phase III ended and phase IV began (min)	197 ± 48	188 ± 57	.51
FFA level at 360 min (mEq/L)	742 ± 156	732 ± 214	.88

was similar in the 2 protocols (742 ± 156 *v* 732 ± 214 μmol/L, $P = .88$). The final FFA plateau was 171% higher than basal ($P = .001$).

Epinephrine Concentrations

Fasting epinephrine levels in the 2 FSIGT protocols were 6.06 ± 4.80 versus 10.86 ± 4.88 pg/mL, $P = .13$. For the first 25 minutes no significant changes in epinephrine concentrations were detected. Mirroring the 40 to 100 minute sub-basal glycemic period in the IM-FSIGT, epinephrine levels from 60 to 150 minutes were higher in the IM-FSIGT than the GO-FSIGT. At 360 minutes epinephrine levels were similar in the 2 protocols (21.2 ± 8.8 *v* 24.2 ± 17.6 pg/mL, $P = .69$) but 260% ± 167% higher than baseline ($P = .03$).

NS-Studies

Fasting glucose and insulin concentrations were 90.5 ± 3.4 mg/dL and 9.2 ± 9.4 μU/mL, respectively. Throughout the NS-Study, glucose and insulin concentrations varied only slightly from baseline. In contrast, FFA levels remained near basal (388 ± 118 μmol/L) until 180 minutes and then began a slow steady ascent reaching 618 ± 258 μmol/L at 360 minutes (Table 2). Importantly, from 180 to 360 minutes there was no significant difference in FFA levels in the NS-Study and IM-FSIGT (Table 2). This suggests that the suprabasal FFA plateau in phase IV is independent of the dynamic changes in insulin and glucose that occur in the FSIGT protocol.

DISCUSSION

The purpose of this investigation was to evaluate the FFA profile during both the GO-FSIGT and the IM-FSIGT. In the GO-FSIGT the only insulin provided is from endogenous secretion. In the IM-FSIGT there is sequential exposure to both endogenous and exogenous insulin. Because of the large differences in the FSIGT protocols in glucose, insulin and epinephrine concentrations, we anticipated that FFA levels would also differ. We postulated that by identifying the differences in the FFA profiles between the protocols, we could localize points at which insulin regulation of FFA was occurring. Indeed, examination of the FFA profile during the FSIGT revealed 4 distinct phases, which were similar in the 2 protocols. As endogenous insulin secretion was essentially identical between protocols, our results suggest that endogenous insulin maximally inhibits lipolysis, and that exogenous insulin has no additional effect on FFA dynamics.

Phase I

In both FSIGT protocols, the intravenous injection of glucose led to the endogenous secretion of insulin. As adipocyte lipolysis is more sensitive to insulin than skeletal muscle glucose uptake,[14,15] we expected FFA to decline rapidly after the endogenous insulin peaked. However, despite hyperglycemia and hyperinsulinemia FFA concentration remained virtually unchanged at fasting levels ~10 minutes (phase I). Zoratti et al also reported in FSIGTs a refractory delay in the decline of FFA after glucose injection.[16] In their experiments the delay was 30 minutes. The reason for the difference in the duration of this "refractory" period between the 2 investigations is unclear. Yet finding a refractory period in both investigations supports its existence.

One possible explanation for this refractory period is transendothelial delay in the transport of insulin to the interstitium.[17,18] By analogy the rate of glucose uptake by muscle is limited by the time it takes for insulin to cross the endothelial barrier and bind to insulin-sensitive cells.[17] In humans this delay can last several hours.[19] However, the delay in suppression of FFA in the present studies was less. However, the exquisite sensitivity of the adipocyte to insulin suggests that total inhibition of lipolysis would occur at a much lower interstitial insulin concentration that that required for stimulation of glucose uptake. Therefore, the approximate 10-minute delay before FFA levels decline may represent the time re-

Table 2. FFA Concentrations at Baseline and During Phase IV in the NS-Study and IM-FSIGT

	Time							
	0 min	180 min	210 min	240 min	270 min	300 min	330 min	360 min
NS-Study (n = 6)	388 ± 118	402 ± 189	409 ± 185	470 ± 185	510 ± 181	544 ± 204	584 ± 225	618 ± 258
IM-FSIGT (n = 6)	383 ± 154	391 ± 143	553 ± 182	618 ± 220	637 ± 186	686 ± 236	703 ± 221	719 ± 221
P value	.90	.88	.15	.08	.11	.09	.23	.33

quired for interstitial insulin to reach the concentration necessary to initiate suppression of lipolysis.

An alternative mechanism for the delay in FFA suppression could be the time it takes for recruitment of insulin sensitive capillary networks in adipose tissue. Vincent et al have shown that insulin appears to recruit "nutritive" capillaries.[20] Hence, insulin might "recruit" capillaries in adipose tissue beds, which are more sensitive to insulin than muscle capillaries. Also, the time required for insulin-induced inhibition of hormone-sensitive lipase (HSL) to occur is unknown. Therefore, a delay in both insulin transport and signaling could contribute to the duration of phase I.

Phase II

The decline in FFA levels during phase II is consistent with a total block in lipolysis caused by the inhibition of HSL by endogenous insulin.[21] However, the transient hyperglycemia caused by the glucose injection at baseline may also contribute to the decrease in lipolysis.[22]

Despite the range of AIRg in the subjects (24 to 2,164 mU/L · min), the addition of exogenous insulin did not appear to influence FFA levels in any subject. The sequential exposure to insulin in the IM-FSIGT is analogous to a stepwise insulin clamp. Low-dose stepwise insulin clamp studies have demonstrated that once insulin has maximally suppressed FFA concentration, further increases in insulin concentration do not lead to further changes in plasma FFA.[6] Evidence in this investigation that endogenous insulin maximally suppressed FFA and that there was no additional effect of exogenous insulin is 3-fold: first, the characteristics of the FFA profile in the GO-FSIGT and IM-FSIGT were similar; second, in the IM-FSIGT the maximum rate of decline of FFA was attained before the exogenous insulin was administered; third, in the IM-FSIGT the rate of decline of FFA did not differ before and after the insulin infusion. But because endogenous insulin led to complete suppression of FFA, the dose-response relationship between endogenous insulin and FFA suppression cannot be elucidated in this study. Administering a lower dose of glucose may make it possible to define the dose-response relationship between endogenous insulin and FFA suppression.

Phase III

The FFA nadir occurred at approximately 80 minutes. Then FFA levels started to rise, suggesting a resumption of lipolysis. As phase III commences, plasma insulin has already declined to basal and epinephrine concentration is increasing. That the increase in lipolysis was delayed beyond the decrease in plasma insulin supports the hypothesis that the effects of insulin on FFA are slowed by the time it takes for insulin to access the interstitial compartment. There is a close relationship between interstitial, but not plasma insulin and the rate of glucose uptake.[23] Thus, even though plasma insulin is normalized while FFA are increasing, it is likely that interstitial insulin in adipose interstitial fluid exceeds the plasma concentration[23] and continues to exert an ever decreasing inhibitory action on lipolysis as the FFA levels are going up during phase III.

Importantly, the rate of rise of FFA during phase III was similar in the GO-FSIGT and IM-FSIGT (5.11 ± 2.99 *v* 4.14 ± 2.15 μmol/L · min, $P = .27$). Theoretically if interstitial insulin levels are greater in the IM-FSIGT than the GO-FSIGT, this should lead to a slower rate of rise of FFA in the IM-FSIGT. However, glucose concentrations are also significantly lower in the IM-FSIGT from 40 to 100 minutes and the epinephrine response from 60 to 150 minutes is significantly higher. Hence the balance of the opposing effects of insulin and epinephrine on HSL appears to result in similar rates of lipolysis in the 2 protocols.

Phase IV

Phase IV began at similar times in the 2 protocols (197 ± 48 *v* 188 ± 57 minutes, $P = .51$). By 360 minutes in both protocols FFA levels plateaued at a concentration 171% ± 107% of basal. We were able to detect this phase, because the FSIGTs were of 360 minutes duration. FSIGTs of 180 minutes are of sufficient duration to calculate S_I,[11] but at 180 minutes, FFA levels are still rising.[16] Therefore we extended our FSIGTs and found that during phase IV, FFA levels plateaued at a new steady-state.

Diurnal elevation of FFA levels is a well described phenomenon and has been implicated as the cause of the diurnal increase in insulin resistance.[24] To determine if the suprabasal FFA plateau during phase IV was secondary either to the FSIGT protocol per se or secondary to diurnal variation, the NS-Study was performed. During the NS-Study the FFA levels remained near basal until 180 minutes and then they began a slow steady ascent. Support for diurnal variation as the cause of the suprabasal FFA plateau comes from the observation that FFA concentrations from 180 to 360 minutes were similar in the NS-Study and IM-FSIGT. However, a postsuppression rebound in FFA levels, as has been observed after meals or after exposure to niacin, may also contribute to the supra-basal plateau.[25,26]

Our study was performed in African Americans but we believe the multiphasic FFA pattern during the FSIGT is present in other populations as well. Zorrati et al performed FSIGTs of 180 minutes duration in AfroCarribean, Asian Indian, and Caucasian men.[16] They detected the same general FFA pattern that we observed for the first 3 phases. However, while all populations are likely to have 4 FFA phases during an FSIGT of 360 minutes duration, FFA concentrations and phase duration may differ.

Further, we believe the multiphasic FFA pattern during the FSIGT is independent of sex, BMI, and glucose tolerance status. We have previously obtained FFA levels in 64 subjects who had only IM-FSIGTs.[27] Of these 64 subjects (27 men, 37 women), 27 were obese and 14 were glucose-intolerant, and all had four FFA phases during the IM-FSIGT.

In examining FFA levels during the IM-FSIGT and GO-FSIGT, we observed that 4 FFA phases are present in both protocols in all subjects. Hence, the insulin effect on FFA during the FSIGT can be attributed to endogenous insulin. We suggest that the regulation of FFA by insulin is best isolated during the FSIGT by determining the conditions that allow endogenous insulin to initiate the inhibition of lipolysis and terminate phase I. To gain further insight, decreasing the dose of glucose administered during the FSIGT may have the twin

benefit of allowing an examination of the concentration of endogenous insulin required to initiate the inhibition of lipolysis and allow the determination of a dose-response relationship between endogenous insulin and FFA suppression.

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