Effects of Insulin on Fatty Acid Reesterification in Healthy Subjects

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The effects of insulin on triacylglycerol/fatty acid cycling (fatty acid reesterification) were studied in 12 normal subjects during euglycemic hyperinsulinemia with the use of stable isotope dilution analysis ([$^2H_5$]glycerol and [1-^{13}C]palmitate) in combination with indirect calorimetry. During basal conditions, 5.6 ± 0.6 μmol · kg^{-1} · min^{-1} of fatty acid were released of which ~3.3 μmol · kg^{-1} · min^{-1} were oxidized and ~2.2 μmol · kg^{-1} · min^{-1} were reesterified. A minority of the recycled fatty acid, (0.8 ± 0.4 μmol · kg^{-1} · min^{-1}) never left the intracellular space before being reesterified (intracellular triacylglycerol/fatty acid cycling), whereas the majority (1.2 ± 0.4 μmol · kg^{-1} · min^{-1}) were first released into the extracellular space and then reesterified in various organs (extracellular triacylglycerol/fatty acid cycling). In response to insulin, fatty acid release declined by 71% (from 5.6 ± 0.6 to 1.6 ± 0.2 μmol · kg^{-1} · min^{-1}). Fatty acid oxidation (measured by indirect calorimetry) declined by 55% (from 3.3 ± 0.3 to 1.5 ± 0.3 μmol · kg^{-1} · min^{-1}) and total triacylglycerol/fatty acid cycling was completely suppressed (from 2.2 to 0.0 μmol · kg^{-1} · min^{-1}). Fatty acid release, oxidation, total and extracellular triacylglycerol/fatty acid cycling all correlated positively with plasma fatty acid concentrations. These data showed that insulin profoundly suppressed fatty acid release, oxidation as well as reesterification of those fatty acids that had entered the extracellular compartment. They suggested that physiological concentrations of insulin suppressed extracellular fatty acid reesterification primarily by inhibiting lipolysis. *Diabetes* 42:1588–93, 1993

Elevated plasma concentrations of FAs have been shown to suppress carbohydrate uptake and oxidation in healthy subjects (1) and have been proposed as a key factor in the pathogenesis of NIDDM (2). It has, therefore, become important to understand better the mechanisms that control plasma FA concentrations. The process of FA release (lipolysis) has been studied extensively (3), but little is known about the regulation of FA reesterification, also called TG/FA cycling, which together with FA oxidation accounts for the clearance of FA from plasma.

Two types of TG/FA cycling have been recognized. FA can be reesterified without leaving the IC space. This has been referred to as IC TG/FA cycling (4–6). Alternatively, FA can enter the EC compartment to be reesterified subsequently in various tissues including liver and muscle. This has been referred to as EC TG/FA cycling (6). Evidence suggests that both forms of FA reesterification in human subjects is under hormonal and substrate control. Wolfe et al. (6) have demonstrated that hyperglycemia associated with hyperinsulinemia increased IC and decreased EC TG/FA cycling. Miyoshi et al. (7) have shown that EPI increased IC cycling. The role of insulin, the most important antilipolytic hormone in humans (3) in IC and EC TG/FA cycling, has not been investigated to our knowledge. We have, therefore, investigated effects of insulin (during euglycemic-hyperinsulinemic clamping) on lipolysis, FA oxidation, and total, IC, and EC reesterification in 12 normal, healthy volunteers using the stable isotopes [$^2H_5$]glycerol and [1-^{13}C]palmitate in combination with indirect calorimetry.

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TG, triacylglycerol; FA, fatty acid; NIDDM, non-insulin-dependent diabetes mellitus; IC, intracellular; EC, extracellular; EPI, epinephrine; NE, norepinephrine; APE, atom percent excess; IVGTT; intravenous glucose tolerance test; m/e, mass/charge ratio; FFM, fat-free mass; BMI, body mass index; R_a, rate of appearance; RIA, radioimmunoassay; MANOVA, multiple analysis of variance; FFA, free fatty acid; GIR, glucose infusion rate.

TABLE 1
Clinical characteristics of study subjects

Sex (M/F)	11/1
Age (yr)	49 ± 5
Height (cm)	170.4 ± 2.6
Weight (kg)	78.2 ± 3
BMI (kg/m^2)	26.8 ± 1.2
Total body fat (kg)	17.6 ± 2.4
Fat (% body wt)	22.0 ± 2.4
FFM (kg)	60.5 ± 2.2
FFM (% body wt)	78.1 ± 2.4

Data are means ± SE.

RESEARCH DESIGN AND METHODS

We have studied 12 healthy subjects (1 woman and 11 men) at 49 ± 5 yr of age. Characteristics of the study group are listed in Table 1. All subjects were in good health and excellent physical condition. None were taking any medications. All led physically active lives, but only one participated regularly in sports. All had normal glucose tolerance as determined by IVGTTs (mean coefficient of glucose disappearance: 2.0, range 1.1–3.6; normal >1.0). The weights of all subjects had been stable for at least 2 mo, and their diets contained a minimum of 250 g/day of carbohydrates for at least 2 days before studies. Informed consent was obtained from all after explanation of the nature, purpose, and potential risks of the study. The study protocol was approved by the Institutional Review Board for Human Research of Temple University.

All study subjects were admitted on the evening before the studies to the General Clinical Research Center at Temple University Hospital. After an overnight fast, euglycemic-hyperinsulinemic clamps were performed in combination with infusion of stable isotopes for measurement of glycerol and palmitate turnover and with indirect calorimetry for estimation of rates of net lipid oxidation. A short polyethylene catheter was inserted into an antecubital vein for infusion of test substances, a second catheter was inserted into a contralateral forearm vein for blood sampling. This arm was wrapped in a heating blanket to arterialize venous blood.

Euglycemic-hyperinsulinemic clamp. Regular human insulin (Humulin R, Lilly, Indianapolis, IN) was infused intravenously at 6 pmol · kg^{-1} · min^{-1} for 4 h starting at 0 min. Glucose concentrations were clamped at ~4.7 mM by a feedback controlled infusion of 20% dextrose. Blood glucose concentrations were determined every 5–10 min with a Beckman glucose analyzer (Palo Alto, CA), and glucose infusions were adjusted accordingly.

Indirect calorimetry. Respiratory gas exchange rates were determined as described previously at 30-min intervals with a metabolic measurement cart (Beckman) (8). Rates of protein oxidation were estimated from urinary nitrogen excretion after correction for changes in urea nitrogen pool size (9). Rates of protein oxidation were used to determine the nonprotein respiratory quotient and net lipid oxidation.

Body composition. Body fat mass was determined by underwater weighing in a water tank with corrections for simultaneously measured residual lung volume (10). Residual lung volume was estimated after immersion in a sitting position by means of a closed-circuit 0_2 dilution method (10).

Glycerol and palmitate turnovers. [$^2H_5$]glycerol (98 atom percent deuterium, Tracer Technologies, Somerville, MA) dissolved in normal saline was infused for 5.5 h (−90–240 min) starting with a priming dose of 1.6 μmol/kg followed by a continuous infusion of 0.11 μmol · kg^{-1} ·min^{-1}. [1-^{13}C]palmitic acid (99.5 atom percent 1-^{13}C, Tracer Technologies) was bound to albumin as described by Wolfe (11) and given by continuous intravenous infusion at a rate of 0.04 μmol · kg^{-1} · min^{-1} for 5.5 h (−90–240 min). Blood for determination of [$^2H_5$]glycerol and [1-^{13}C]palmitate enrichment was collected at 30-min intervals from before the start (−90 min) until the end (240 min) of the clamp. Plasma was immediately separated at 4°C and stored at −20°C until analyzed. The trimethylsilyl derivative of glycerol was prepared as described previously (12). [$^2H_5$]glycerol enrichment was determined by gas chromatography-mass spectrometry (model 4610-B, Finnigan-Matt, San Jose, CA) with the use of electron impact ionization and monitoring of ions at m/e 205 and 208. Plasma FAs were extracted and isolated by thin-layer chromatography. Methylester derivatives of [1-^{13}C]palmitic acid were prepared, and [1-^{13}C]palmitate enrichment was determined by gas chromatography-mass spectrometry at m/e 270 and 271 as described previously (13).

Measurements of individual FA. Myristate (C14), palmitate (C16), palmitoleate (C16:1), stearate (C18), oleate (C18:1), linoleate (C18:2), and arachidonate (C20:4) were determined by gas chromatography (model 5730A, Hewlett-Packard, Avondale, PA) with the use of heptadecanoic acid (C17) as internal standard.

Calculations. Because [$^2H_5$]glycerol and [1-^{13}C]palmitate enrichments were stable before (−30–0 min) and at the end of the clamp (210–240 min) (Fig. 1), the R_a of glycerol and palmitate were calculated according to the steady-state equation of Steele corrected for the amount of exogenously infused stable isotope (14)

$$R_a = (IE_{inf}/IE_{pla} - 1) \times F$$

where R_a is the rate of appearance of glycerol or palmitate (μmol · kg^{-1} · min^{-1}), IE_{inf} is the isotopic enrichment of the infusate (APE), IE_{pla} is the isotopic enrichment of plasma (APE) at isotopic equilibrium, and F is the isotope rate of infusion (μmol · kg^{-1} · min^{-1}).

Glycerol R_a × 3 was assumed to reflect rates of whole-body lipolysis. The validity of this assumption is supported by the following evidence. *1*) Glycerol is only produced by lipolysis (6,15). *2*) Because of a virtual absence of α-glycerol kinase in adipose tissue and muscle, all glycerol released by lipolysis will appear in plasma (5). *3*) Splanchnic release of glycerol under basal and hyperinsulinemic conditions is minimal and thus underestimation of glycerol R_a because of first-pass hepatic clearance is not a problem (16). *4*) Underestimation of lipolysis because of partial hydrolysis of triacylglycerol is unlikely (5,17).

FA R_a was calculated by dividing the palmitate R_a by

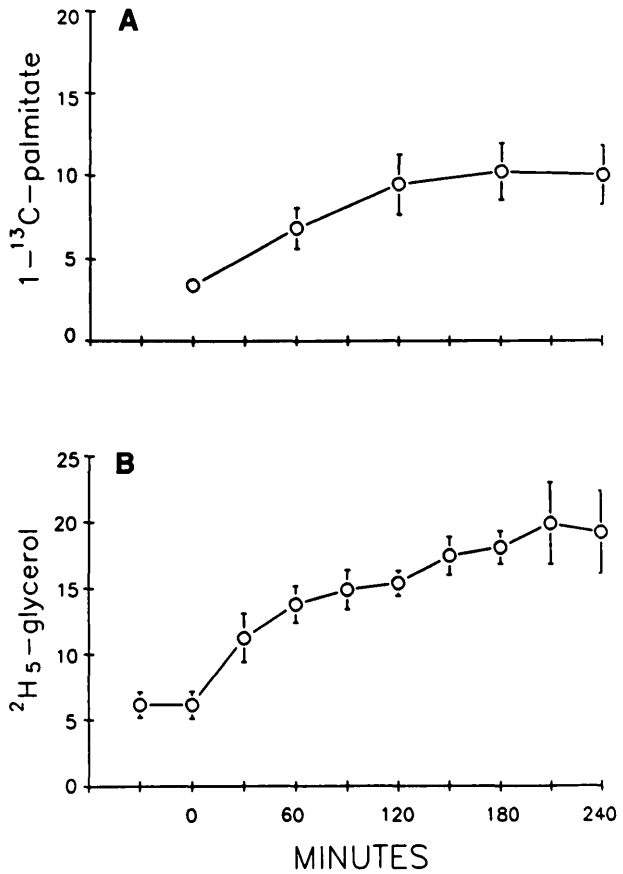

FIG. 1. APE of [1-^{13}C]palmitate (*A*) and [$^{2}H_5$]glycerol (*B*) in 12 healthy subjects during euglycemic clamping. Data are means ± SE.

the fractional contribution of palmitate to the total plasma FA concentration, which was determined by gas chromatographic determination of seven individual FAs

$$\frac{FA_{Ra} = \text{palmitate } R_a}{\text{[palmitate]/[sum of 7 FA]}}$$

FA oxidation was determined by converting the rate of triglyceride oxidation (mg · kg^{-1} · min^{-1}) obtained by indirect calorimetry to its molar equivalent (μmol · kg^{-1} · min^{-1}) with the use of 860 g/mol as the average molecular weight of triglyceride and multiplying the molar rate of triglyceride oxidation by 3, because each mole of TG contains 3 mol of FAs.

TG/FA cycling. During steady-state conditions, the difference between the rate of lipolysis (glycerol R_a × 3) and the rate of FA oxidation provides an index of the rate of total TG/FA cycling, because recycling is ultimately the fate of all nonoxidized FAs (6). Thus, total TG/FA cycling = glycerol R_a × 3 − FA oxidation.

During lipolysis, 3 mol of FA are released for each mole of glycerol; therefore, the difference between glycerol R_a × 3 and FA R_a provides an index of IC TG/FA cycling. It was calculated as follows: IC TG/FA cycling = glycerol R_a × 3 − FA R_a. EC TG/FA cycling was obtained as the difference between total and IC TG/FA cycling.

Analytical procedures. Plasma glucose was measured with a Beckman glucose analyzer. Serum insulin was measured by RIA (18), plasma catecholamines were measured radioenzymatically (19), plasma urea nitrogen (20), glycerol, and TG were measured colorimetrically (21). Urinary nitrogen was measured by the method of Kjeldahl (22). Plasma FAs were determined according to Lorch and Gey (23) after extraction according to Dole and Meinertz (24).

Statistical analysis. All data are expressed as means ± SE. Statistical significances were assessed using MANOVA and two-tailed Student's *t* test.

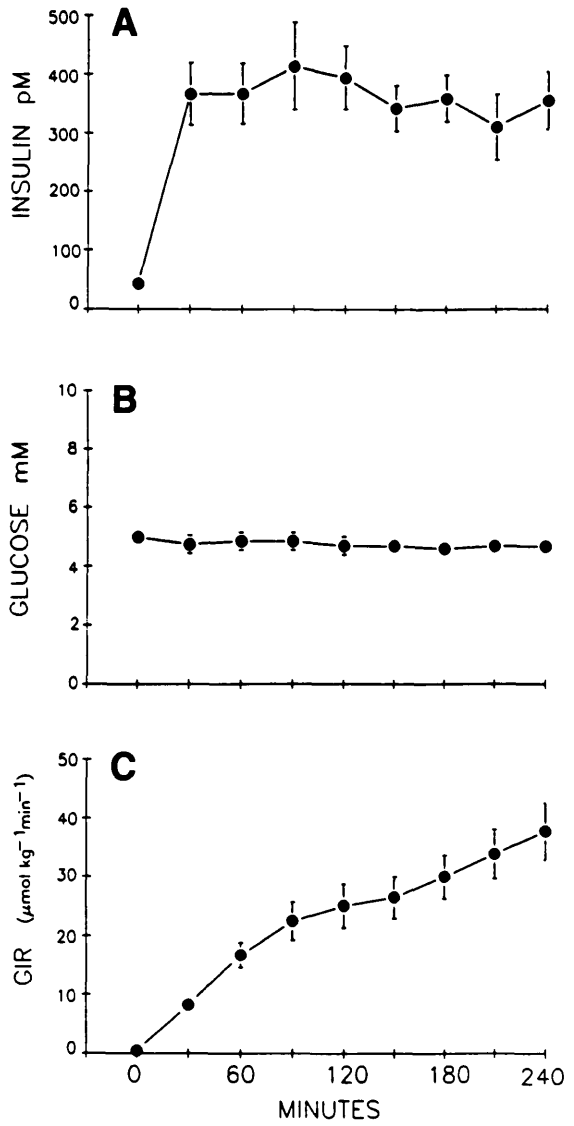

FIG. 2. Serum insulin (*A*), glucose concentrations (*B*), and glucose infusion rates (*C*) during euglycemic-hyperinsulinemic clamping in 12 healthy subjects. Data are means ± SE.

RESULTS

Euglycemic-hyperinsulinemic clamps. Basal serum insulin concentration was 47 ± 10 pM. Mean steady-state clamp insulin concentration was 366 ± 18 pM (Fig. 2). Plasma glucose concentrations were clamped at 4.7 ± 0.05 mM. The glucose infusion rate needed to maintain euglycemia increased from 0 to 37.8 ± 5.2 μmol · kg^{-1} · min^{-1}.

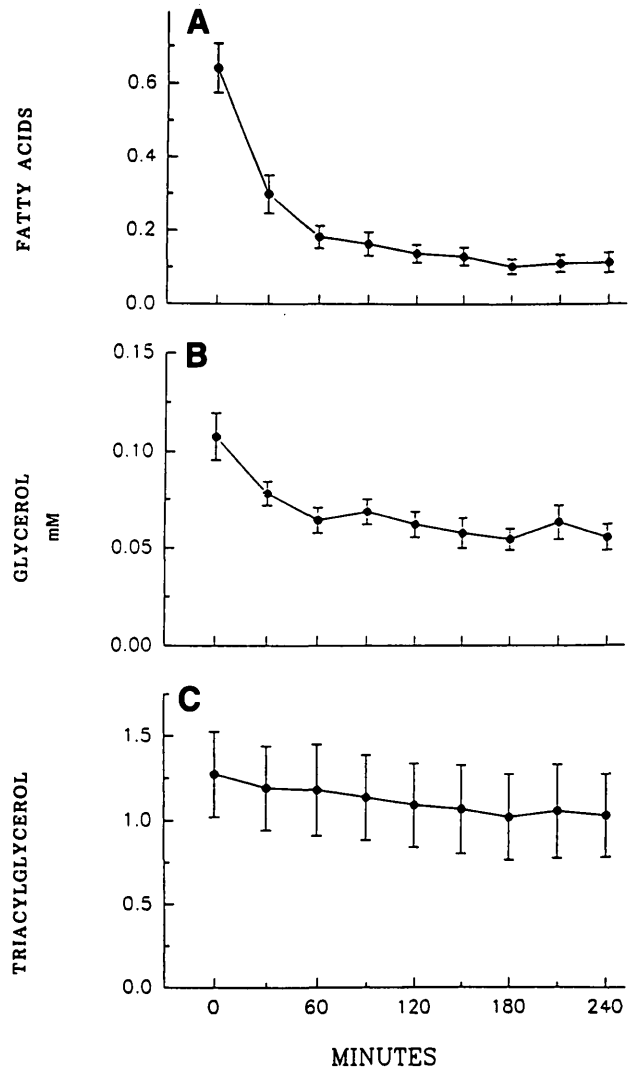

FIG. 3. Plasma concentrations of FA (*A*), glycerol (*B*), and TG (*C*) during euglycemic-hyperinsulinemic clamping in 12 healthy subjects. Insulin (6 pmol · kg^{-1} · min^{-1}) was infused from 0 to 240 min. Data are means ± SE. Statistical evaluation (paired Student's *t* test): FA and glycerol, $P < 0.01$ comparing 0 min with all values between 30 and 240 min; TG, $P < 0.01$ comparing 0 with 240 min.

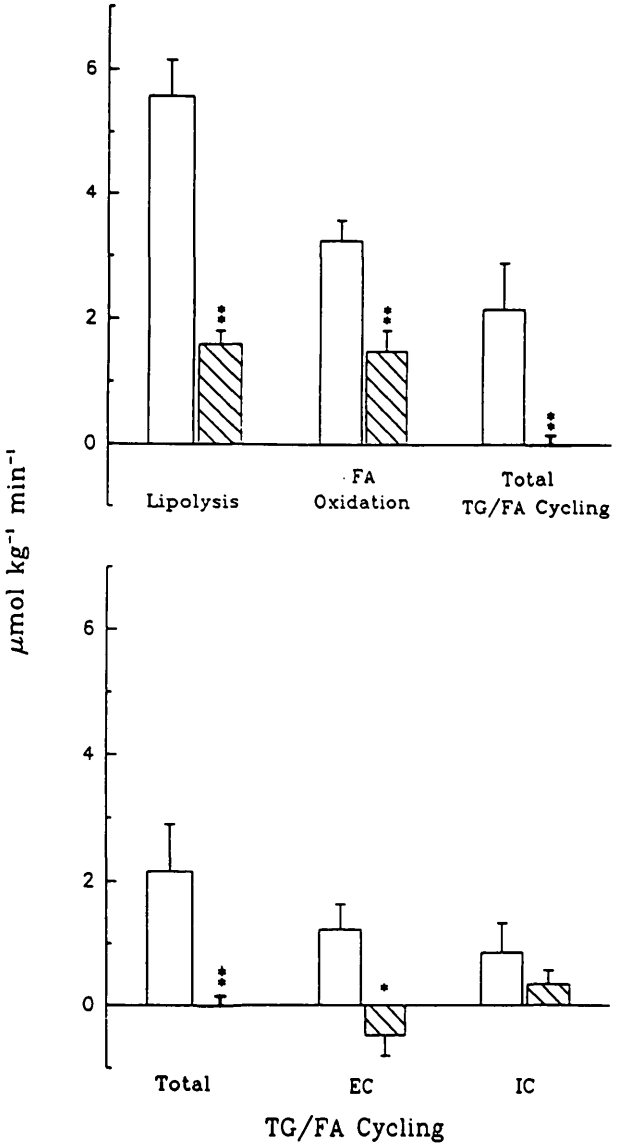

FIG. 4. Rates of lipolysis, FA oxidation (by indirect calorimetry), total, EC, and IC TG/FA cycling in 12 healthy subjects before (□) and after (▨) 4 h of euglycemic hyperinsulinemia. Data are means ± SE. *P < 0.05, **P < 0.01 comparing pre- and postinsulin values.

Plasma lipids. Insulin reduced FA concentrations by 83% from 640 ± 66 to 110 ± 27 μM ($P < 0.001$) (Fig. 3). Insulin had no effect on the percentage contribution of palmitate to total FA (23.6 ± 1.2 before insulin vs. 26.0 ± 2.3% after insulin, NS). Insulin decreased glycerol concentration by 48% from 108 ± 12 to 56 ± 7 μM ($P < 0.001$) and TG concentration by 19% from 1.27 ± 0.25 to 1.03 ± 0.25 mM ($P < 0.01$).

Plasma catecholamines. Plasma EPI and NE concentrations were 186 ± 22 and 1387 ± 176 pM, respectively, before and 196 ± 44 and 1412 ± 150 pM, respectively, at the end of the insulin infusions. The differences between pre- and postinsulin values were not statistically significant.

Glycerol and palmitate turnover. Basal turnover rates of glycerol and palmitate were 1.86 ± 0.19 and 1.23 ± 0.11 μmol · min^{-1} · kg^{-1}, respectively. Insulin suppressed both glycerol and palmitate turnover by 72% to 0.53 ± 0.07 and 0.34 ± 0.09 μmol · min^{-1} · kg^{-1}, respectively.

Lipolysis, FA oxidation, and TG/FA cycling. The basal lipolytic rate was 5.6 ± 0.6 μmol · kg^{-1} · min^{-1} (Fig. 4). Insulin decreased lipolysis by 71% to 1.6 ± 0.2 μmol · kg^{-1} · min^{-1} ($P < 0.01$). Lipolysis correlated closely with plasma FA concentrations ($r = 0.86$, $P < 0.001$) (Table 2) and with FA oxidation ($r = 0.58$, $P < 0.01$).

The basal nonprotein respiratory quotient was 0.78 ± 0.02 and increased to 0.90 ± 0.02 during insulin infusion.

The basal net FA oxidation rate was 3.3 ± 0.3 μmol · kg^{-1} · min^{-1} and decreased by 55% to 1.5 ± 0.3 μmol · kg^{-1} · min^{-1} ($P < 0.01$) in response to insulin. FA oxidation correlated well with plasma FA concentration ($r = 0.74$, $P < 0.001$) but not with IC TG/FA cycling ($r = -0.07$, NS) (Table 2).

TABLE 2
Correlation of data

	FA release		FA oxidation		TG/FA cycling					
					Total		IC		EC	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
FA concentration	0.86	<0.001	0.74	<0.001	0.55	<0.01	0.13	NS	0.64	<0.001
FA release			0.58	<0.01			0.45	<0.05		
FA oxidation							−0.07	NS		

$P > 0.05$ is NS.

The basal rate of total TG/FA cycling was 2.2 ± 0.7 μmol · kg^{-1} · min^{-1}. In response to insulin it decreased to 0.0 ± 0.2 μmol · kg^{-1} · min^{-1} ($P < 0.01$) (Table 3).

The basal rate of IC TG/FA cycling was 0.8 ± 0.4 μmol · kg^{-1} · min^{-1} and did not change significantly during insulin infusion (0.3 ± 0.2 μmol · kg^{-1} · min^{-1}, NS). IC TG/FA cycling did not correlate with plasma FA concentration ($r = 0.13$, NS).

The basal rate of EC TG/FA cycling was 1.2 ± 0.4 μmol · kg^{-1} · min^{-1} and decreased in response to insulin to −0.5 ± 0.3 μmol · kg^{-1} · min^{-1} ($P < 0.05$). EC TG/FA cycling correlated well with plasma FA concentrations ($r = 0.64$, $P < 0.001$).

DISCUSSION

Basal state. In this study, we found that after an overnight fast, 5.6 μmol · kg^{-1} · min^{-1} of FA were released by lipolysis of which 3.3 ± 0.3 μmol · kg^{-1} · min^{-1} (59%) were oxidized, whereas the remainder (~2.2 μmol · kg^{-1} · min^{-1}) was reesterified. Most (~55%) of this recycling took place after the FA had entered the plasma (EC TG/FA cycling). The rest occurred without the FA ever leaving the IC space (IC TG/FA cycling). These results are in general agreement with those from several other laboratories indicating that a large part of FFA released through lipolysis (40–70%) is recycled into TG (25–27) and with data published by Wolfe et al. (6), who found rates of basal (postabsorptive) IC and EC TG/FA cycling of 0.91 ± 0.45 and 1.54 ± 0.5 μmol · kg^{-1} · min^{-1}, respectively, in 8 male volunteers, and Miyoshi et al. (7), who reported postabsorptive IC TG/FA cycling rates ranging from 0.4 to 1.2 μmol · kg^{-1} · min^{-1}. In concordance with these researchers, we found basal IC TG/FA cycling rates to be >20% of lipolytic rates. In fact, true IC TG/FA cycling rates may be even lower because the calculations assumed that all FAs that were lipolyzed but did not enter the EC pool were reesterified. This overestimated IC TG/FA cycling by the amount of FAs that were oxidized before mixing with the glycerol tracer. EC TG/FA cycling was underestimated by the same amount because it was calculated as total minus IC TG/FA cycling.

The basal plasma glycerol concentrations in our subjects (108 ± 12 μM) were ~40% higher than values reported by others (6,25,28). Because glycerol R_as in our subjects were normal (1.86 ± 0.19 μmol · kg^{-1} · min^{-1}), the elevated plasma levels suggested that glycerol clearance was reduced. The reason for this is not clear but may be because our subjects were older (49 ± 5 yr) and had more body fat (22 ± 2.4%). At least one other study has found that obesity was associated with elevated basal glycerol concentrations (29). On the other hand, it is possible that the differences in glycerol levels were related to methodological differences. We measured glycerol enzymatically, whereas most of the lower glycerol values reported in the literature were obtained by gas chromatography.

Effect of insulin. Because glucose has been reported to alter TG/FA cycling (6) and lipolysis (28), it was necessary to determine the effect of insulin on TG/FA cycling during euglycemic hyperinsulinemia. The insulin concentrations attained in our study (~360 pM) were well within the postprandial range and have been shown to suppress lipolysis maximally but not completely (25).

Our results indicated that insulin suppressed lipolysis profoundly (from 3.3 to 1.5 μmol · kg^{-1} · min^{-1}), confirming numerous reports by others (25,30–33). In addition, our data indicated that insulin completely suppressed FA reesterification, affecting exclusively EC TG/FA cycling. The mechanism by which insulin suppressed TG/FA cycling was not investigated. Examining the relationships among TG/FA cycling, plasma FA concentrations, and

TABLE 3
Fatty acid metabolism

	FA release	FA oxidation	FA reesterification		
			Total	IC	EC
Basal (μmol · kg^{-1} · min^{-1})	5.6 ± 0.6	3.3 ± 0.3	2.2 ± 0.7	0.8 ± 0.4	1.2 ± 0.4
Insulin (μmol · kg^{-1} · min^{-1})	1.6 ± 0.2	1.5 ± 0.3	0.0 ± 0.2	0.3 ± 0.2*	−0.5 ± 0.3*
P	<0.001	<0.01	<0.01	NS	<0.05

Data are means ± SE.
*Values not significantly different from 0.

oxidation, however, provided some clues. EC TG/FA cycling correlated closely with plasma FA concentrations and with lipolysis, suggesting that it may be regulated mainly by plasma FA concentrations that in turn were determined predominantly by the rate of lipolysis (34,35). Of note was that TG/FA cycling did not correlate with FA oxidation, suggesting that these two processes were regulated independently from each other. Taken together, the data suggested that insulin suppressed selectively EC TG/FA cycling, probably by inhibiting the release of FA. They did not, however, exclude the possibility that insulin may have also inhibited FA reesterification directly as suggested by Groop et al. (27). These investigators found that insulin decreased net FA oxidation even when plasma FA concentrations were prevented from falling by infusion of heparin.

FA reesterification plays an important role in maintaining FA homeostasis by clearing from the blood those FAs that cannot be oxidized. Its energy cost can be estimated. One mole of recycled FA uses ~8 mol of ATP and ~144 kcal (1 mol of ATP generates ~18 kcal) (36,37). Thus, TG/FA cycling in our subjects consumed ~1.5 kcal/h or ~2.7% of their resting metabolic rate (56 kcal/h).

In summary, this study demonstrated that physiological concentrations of insulin profoundly inhibited FA release, oxidation, and reesterification. Insulin had no effect on IC TG/FA cycling but selectively suppressed EC TG/FA cycling presumably by reducing the amount of FA in plasma available for reesterification.

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