Release, Oxidation, and Reesterification of Fatty Acids From Infused Triglycerides: Effect of Heparin

Xinhua Chen, Jose Ruiz, and Guenther Boden

We have investigated the effects of heparin on rates of fatty acid (FA) release, oxidation, and reesterification from intravenously (IV) infused triglycerides (TGs) during euglycemic (4.7 mmol · L^{-1}) hyperinsulinemia (~450 pmol · L^{-1}). Four healthy men (aged 31 ± 3 years; body mass index, 26.1 ± 0.9 kg/m^2) received IV TGs (1.02 mmol TG · kg^{-1} · $4\ h^{-1}$), four other men (aged 24.3 ± 2.8 years; body mass index, 24.7 ± 1.7 kg/m^2) received TGs plus heparin (200-U bolus followed by 0.4 U · kg^{-1} · min^{-1}), and nine men and one woman (aged 28.8 ± 2.3 years; body mass index, 23.1 ± 0.9 kg/m^2) received saline (controls). Heparin increased lipolysis from infused TGs (to 1.0 ± 0.1 from 0.3 ± 0.1 mmol · kg^{-1} · $4\ h^{-1}$, $P < .01$), increased plasma free fatty acids ([FFA] to 737 ± 32 from 597 ± 136 μmol · L^{-1}, $P < .05$), and increased FA reesterification (to 0.84 ± 0.14 from 0.18 ± 0.12 mmol · kg^{-1} · 4 h^{-1}, $P < .02$), but had no effect on FA oxidation (0.13 ± 0.02 *v* 0.12 ± 0.04 mmol · kg^{-1} · $4\ h^{-1}$) or net energy gain (167 ± 42 *v* 243 ± 79 kJ · $4\ h^{-1}$). In summary, addition of heparin (1) increased lipolysis (to ~98% from ~29%) and reesterification (to ~82% from ~17%) of infused TG, but had no significant effects on fat oxidation (~12%) and net energy gain. We conclude that heparin accelerated removal of infused lipid from the blood and its deposition into endogenous fat depots. Since the doses of heparin and insulin used in this study were higher than those generally used in total parenteral nutrition protocols, our results may not be strictly applicable to the usual clinical situation.

TRIGLYCERIDE (TG) EMULSIONS are commonly used in parenteral nutrition to supply essential fatty acids (FAs) and energy.[1,2] Removal from plasma of these infused TGs has been studied under various conditions,[3-5] and it has been shown that some of the intravenously (IV) administered fat was used as an energy source in patients receiving total parenteral nutrition.[6] However, there is presently no published information on the metabolic fate of infused TGs, ie, the rates at which they are lipolyzed, oxidized, and reesterified in vivo. Moreover, heparin and insulin are frequently infused together with TGs.[1] Heparin, which activates lipoprotein lipase,[7] is given to facilitate utilization of TGs and to prevent hypertriglyceridemia, and insulin is given to facilitate utilization of infused substrates and to prevent hyperglycemia in susceptible individuals.[1,2] It is not known how heparin and insulin affect the metabolism of infused TG emulsions.

It was therefore the objective of the present study (1) to determine lipolysis, oxidation, and reesterification of IV infused TGs in healthy volunteers under conditions of euglycemic hyperinsulinemia, and (2) to study the effect of heparin on these parameters.

SUBJECTS AND METHODS

Subjects

We have studied 18 healthy, normal volunteers. Their ages, body mass indices, and body compositions are shown in Table 1. None of the subjects had a family history of diabetes or any other endocrine disorders, and none were taking any medications. Their weights were stable for at least 2 months, and their diets contained a minimum of 250 g/d carbohydrate for at least 2 days before the studies. Their life-styles were essentially sedentary, and their weekly exercise-related energy expenditure was less than 2,000 kcal. Informed written consent was obtained from all subjects after explanation of the nature, purpose, and potential risks of these studies. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

Experimental Design

All subjects were admitted to the Temple University Hospital General Clinical Research Center on the evening before the studies, which began at approximately 8 AM after an overnight fast and with the subjects reclining in bed. A short polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was placed into a contralateral forearm vein for blood sampling. This arm was wrapped with a heating blanket (~70°C) to arterialize venous blood.

Three different protocols were used. Four subjects received infusions of TGs, four received TGs together with heparin, and 10 received only saline. All studies were performed during euglycemic hyperinsulinemia.

Blood samples were collected at 30-minute intervals for 4 hours, placed immediately on ice, and centrifuged at 4°C. To prevent in vitro lipolysis, diethyl *p*-nitrophenyl phosphate (Paroxam; Sigma Chemicals, St Louis, MO) was added to all blood samples. Plasma was frozen at −20°C until analyzed.

Euglycemic-Hyperinsulinemic Clamps With LIPOSYN II and Heparin

Regular human insulin (Humulin; Eli Lilly, Indianapolis, IN) was infused IV at a rate of 6 pmol · kg^{-1} · min^{-1} for 4 hours starting at 0 minutes. Glucose concentrations were clamped at approximately 4.7 mmol · L^{-1} by a feedback-controlled infusion of 20% dextrose. Blood glucose concentrations were determined every 5 to 10 minutes with a glucose analyzer, and glucose infusions were adjusted accordingly. The infused solution was LIPOSYN II (Abbott Laboratories, N Chicago, IL), a 20% TG solution (10% safflower oil and 10% soybean oil) containing 23 mmol · dL^{-1} glycerol as emulsifier. It was infused at a rate of 1.5 mL · min^{-1} for 4

From the Division of Endocrinology and Metabolism and the General Clinical Research Center, Temple University School of Medicine, Philadelphia, PA.

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Address correspondence to Guenther Boden, MD, Temple University Hospital, 3401 N Broad St, Philadelphia, PA 19140.

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Table 1. Characteristics of Subjects

Characteristic	Lipid	Lipid + Heparin	Saline	P
No.	4	4	10	
Sex (M/F)	4/0	4.0	9/1	
Age (yr)	31.0 ± 3.0	24.3 ± 2.8	28.8 ± 2.3	NS
Body weight (kg)	82.9 ± 4.6	83.3 ± 8.2	70.2 ± 3.7	NS
Height (cm)	177.2 ± 3.6	182.9 ± 2.2	173.8 ± 2.2	NS
BMI (kg/m^2)	26.1 ± 0.9	24.7 ± 1.7	23.1 ± 0.9	NS
Fat mass (%)	15.1 ± 2.9	15.3 ± 2.2	14.5 ± 1.3	NS

NOTE. Values are the mean ± SE.
Abbreviation: BMI, body mass index.

hours starting at 0 minutes. Heparin (0.4 U · kg^{-1} · min^{-1}) was infused IV for 4 hours starting at 0 minutes with a 200-U bolus.

Indirect Calorimetry

Respiratory gas-exchange rates were determined at 30-minute intervals with a metabolic measurement cart (Beckman Instruments, Palo Alto, CA) as described previously.[8,9]

Glycerol Turnover

Glycerol turnover was determined with $^2H_5$-glycerol by gas chromatography—mass spectrometry as described previously.[10]

Measurements of FAs

FAs were determined as the sum of seven individual FAs (myristate, palmitate, palmitoleate, stearate, oleate, linoleate, and arachidonate) by gas chromatography (model 5730A; Hewlett-Packard, Avondale, PA) with heptadecanoic acid (C17) as internal standard.

Body Composition

Body composition was determined by underwater weighing in a water tank, with corrections for simultaneously measured residual lung volume.[11]

Calculations

Enrichments of $^2H_5$-glycerol during lipid and lipid plus heparin infusions were at steady state for the last 3 hours (Fig 1). The rates of appearance (R_a) were therefore calculated according to the steady-state equation of Steele corrected for the amount of exogenously infused isotope.[12]

The R_a of glycerol in control studies was calculated using the non–steady-state equation of Steele.[13] All turnover rates were corrected for the amount of exogenously infused glycerol in LIPOSYN II (glycerol, 5.25 ± 0.33 μmol · kg^{-1} · min^{-1}).

Rates of lipolysis and FA reesterification were calculated as follows. Glycerol R_a times three was assumed to reflect rates of whole-body lipolysis: lipolysis = glycerol R_a × 3. This assumption has been validated extensively.[14-18]

Lipolysis from infused TGs was calculated by subtracting rates of lipolysis in controls (no lipid infusion) from those in subjects who received lipid infusion: lipolysis from infused TG = lipolysis − lipolysis in controls.

Four-hour rates of lipolysis, FA oxidation, and FA reesterification were calculated as areas under the respective curves using the trapezoidal rule.

The difference between the rate of lipolysis and the rate of FA oxidation provides an index of the rate of total FA reesterification, because reesterification is ultimately the fate of all nonoxidized FAs: total reesterification = lipolysis − FA oxidation.

Rates of FA oxidation and reesterification from infused TGs were calculated by subtracting rates of FA oxidation and reesterification in controls from those obtained during lipid plus heparin and lipid studies.

Energy generation from FA oxidation and energy expenditure for FA reesterification from infused TGs were estimated assuming that 1 mol palmitic acid oxidized produced 129 mol adenosine triphosphate (9,715 kJ) and that reesterification of 1 mol FA consumed 602 kJ.[19,20] The net energy gain was the difference between energy generation from FA oxidation and the energy cost of FA reesterification.

Analytical Procedures

Plasma glucose level was measured with a glucose analyzer (Beckman Instruments). Serum insulin level was measured by radioimmunoassay.[21] Plasma urea nitrogen,[22] glycerol, and TG levels were measured colorimetrically.[23] Urinary nitrogen level was measured by the Kjeldahl method.[24]

Statistical Analysis

All data are expressed as the mean ± SEM. Statistical evaluation was performed using BMDP statistical software (1990 version; Los Angeles, CA). ANOVA was performed followed by a Bonferroni test for pairwise comparisons between group means. ANOVA with repeated measures was used to compare the difference between groups. Paired and unpaired *t* tests were used to analyze differences between and within groups.

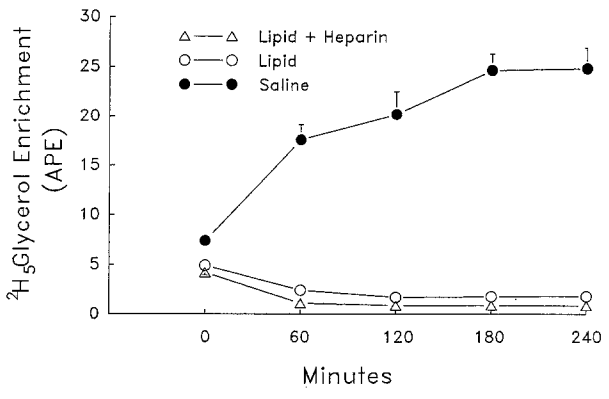

Fig 1. Enrichment of [$^2H_5$]glycerol during infusion of lipid + heparin (n = 4), lipid (n = 4), or saline (n = 10) during euglycemic hyperinsulinemia (mean ± SE).

RESULTS

Euglycemic Clamps

In all three studies, insulin infusions increased plasma insulin concentrations from approximately 30 to approximately 450 pmol · L^{-1} . Plasma glucose was clamped at approximately 4.7 mmol · L^{-1}.

Plasma TGs and Free Fatty Acids

LIPOSYN II infusion increased plasma TGs approximately eightfold from 0.74 ± 0.22 to 6.05 ± 1.79 mmol · L^{-1} ($P < .05$) and plasma free fatty acids (FFA) by approximately 20% from 499 ± 119 to 597 ± 136 μmol · L^{-1} (nonsignificant). LIPOSYN II plus heparin infusion increased plasma TGs approximately 4.5-fold from 0.89 ± 0.21 to 4.0 ± 0.11 mmol · L^{-1} ($P < .001$) and plasma FFA by approximately 77% from 415 ± 69 to 736 ± 32 μmol ·

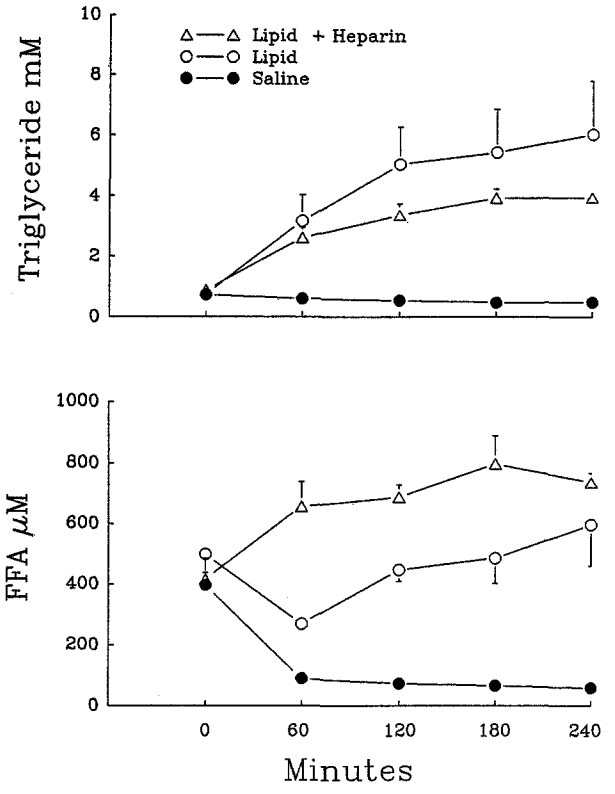

Fig 2. Plasma TG and FFA concentrations during infusion of lipid + heparin (n = 4), lipid (n = 4), or saline (n = 10) during euglycemic hyperinsulinemia. Statistical analysis: FFA, overall comparison of groups Δ v O, $P < .05$, Δ v ●, $P < .001$, and O v ●, $P < .001$; TGs, Δ v O, $P > .05$, Δ v ●, $P < .001$, and O v ●, $P < .001$.

L^{-1} ($P < .001$). Infusion of insulin without LIPOSYN II or heparin (controls) decreased TGs from 0.73 ± 0.13 to 0.49 ± 0.10 mmol · L^{-1} ($P < .001$) and plasma FFA from 397 ± 41 to 59 ± 4 μmol · L^{-1} ($P < .001$) (Fig 2).

Lipolysis and FA Oxidation and Reesterification During Saline Infusions

In controls, rates of lipolysis during euglycemic-hyperinsulinemic clamps decreased from 6.3 ± 0.8 to 1.6 ± 0.2 μmol · kg^{-1} · min^{-1} (glycerol R_a decreased from 2.1 ± 0.2 to 0.53 ± 0.07 μmol · kg^{-1} · min^{-1}, −73%, $P < .001$), rates of FA oxidation decreased from 3.3 ± 0.2 to 1.7 ± 0.4 μmol · kg^{-1} · min^{-1} (−48%, $P < .01$), and rates of FA reesterification decreased from 3.0 ± 0.9 to 0.4 ± 0.1 μmol · kg^{-1} · min^{-1} (−87%, $P < .02$) (Fig 3).

Lipolysis and FA Oxidation and Reesterification From LIPOSYN II

Lipolysis from infused TGs did not become apparent until the second hour (~4.0 μmol · kg^{-1} · min^{-1}) and then increased further to approximately 6.0 μmol · kg^{-1} · min^{-1} (at 4 hours). Addition of heparin accelerated and increased lipolytic rates, which reached a plateau of approximately 17 μmol · kg^{-1} · min^{-1} after about 2 hours (Fig 4).

FA oxidation from infused LIPOSYN II increased from 0.8 ± 0.5 after 1 hour to 2.6 ± 0.7 μmol · kg^{-1} · min^{-1} after 4 hours ($P < .05$). Addition of heparin had no significant additional effect on FA oxidation from infused TGs, which increased from 0.8 ± 0.4 after 1 hour to 3.4 ± 0.3 μmol · kg^{-1} · min^{-1} after 4 hours ($P < .05$).

Total reesterification from infused TGs slowly increased to reach 4.0 ± 2.0 μmol · kg^{-1} · kg^{-1} · min^{-1} after 4 hours. Addition of heparin significantly increased total reesterification from 7.6 ± 2.5 after 1 hour to 13.5 ± 4.6 μmol · kg^{-1} · min^{-1} after 4 hours ($P < .05$).

Metabolism of Infused TGs

The fate of infused TGs is summarized in Table 2. The same amount of TGs (~1 mmol · kg^{-1} · 4 h^{-1}) was infused in lipid and lipid plus heparin studies. Of this amount, 98% and 29%, respectively, were lipolyzed in the lipid plus

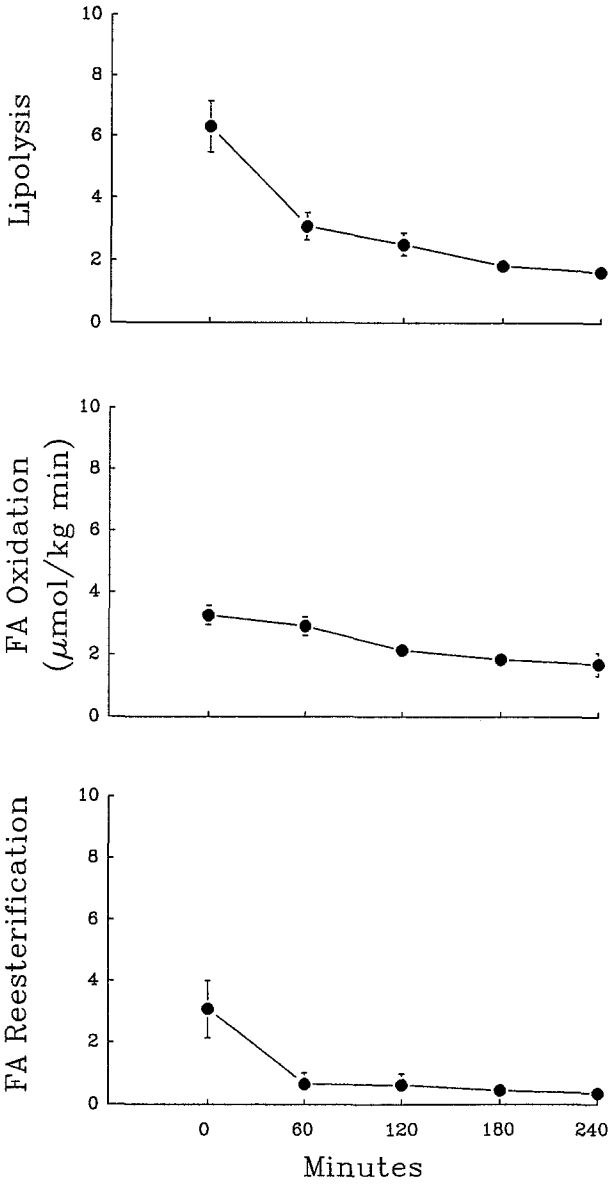

Fig 3. Lipolysis, FA oxidation, and FA reesterification during euglycemic-hyperinsulinemic clamps in 10 healthy volunteers.

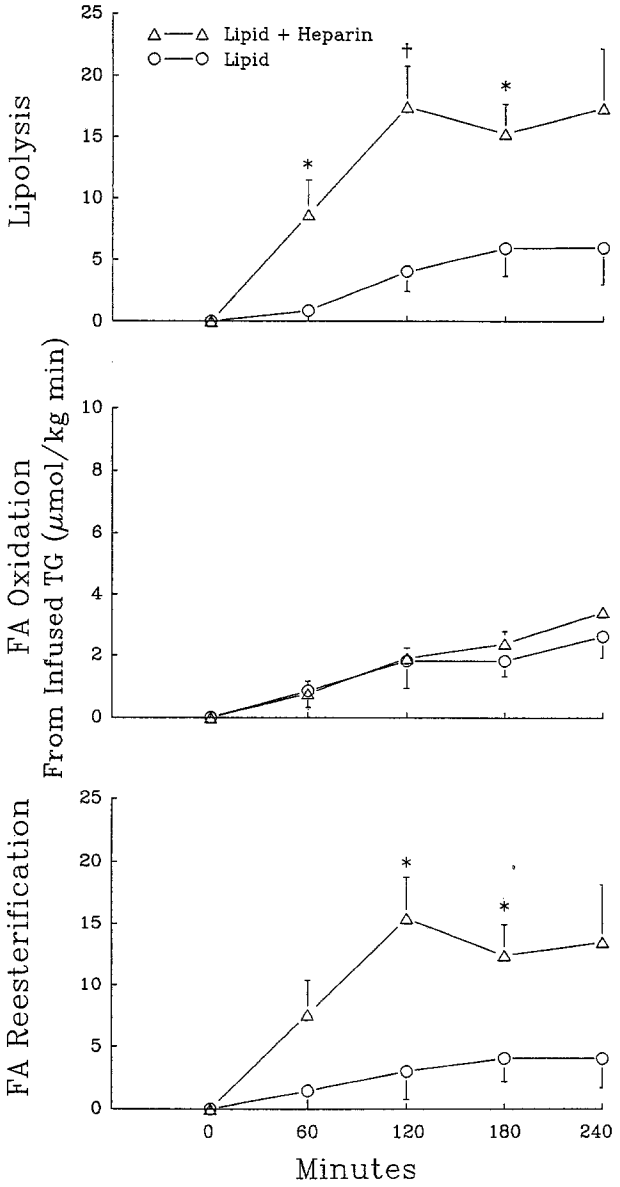

Fig 4. Lipolysis, FA oxidation, and FA reesterification from infused TGs during infusion of lipid + heparin or lipid during euglycemic hyperinsulinemia. Statistical analysis: lipolysis, overall comparison of curves, $P < .05$; comparing individual time points at 60, 120, and 180 minutes, $*P < .05$, $\dagger P < .02$; FA reesterification, overall comparison of curves, $P < .05$; comparing individual time points at 120 and 180 minutes, $*P < .05$.

heparin and lipid studies ($P < .01$) and 82% and 17%, respectively, were reesterified ($P < .05$), whereas oxidation rates were the same in both groups (12%, or 126 ± 24 and 120 ± 37 μmol · kg^{-1} · 4 h^{-1}, respectively).

Table 2. Metabolism of Infused TGs

TGs	Heparin (μmol · kg^{-1} · 4 h^{-1})	No Heparin (μmol · kg^{-1} · 4 h^{-1})	P
Infused	1,034 ± 98 (100%)	1,020 ± 63 (100%)	NS
Lipolyzed	1,018 ± 134 (98%)	296 ± 109 (29%)	<.01
Oxidized	126 ± 24 (12%)	120 ± 37 (12%)	NS
Reesterified	843 ± 138 (82%)	176 ± 117 (17%)	<.02

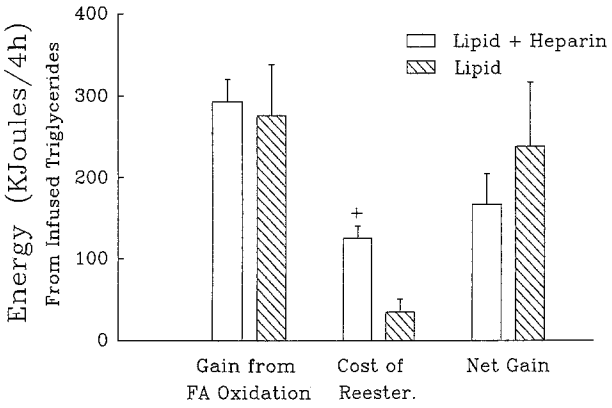

Fig 5. Energy balance over 4 hours of infusions with lipid + heparin or with lipid. $\dagger P < .02$, lipid + heparin *v* lipid infusions.

Energy Balance

Oxidation of infused TGs generated 276 ± 63 kJ · 4 h^{-1} at a cost for reesterification of 33 ± 17 kJ · 4 h^{-1}, for a net gain of 243 ± 79 kJ · 4 h^{-1}. Addition of heparin produced 293 ± 29 kJ · 4 h^{-1} at a cost of 126 ± 17 kJ · 4 h^{-1}, for a net gain of 167 ± 42 kJ · 4 h^{-1} (Fig 5).

DISCUSSION

In the present study, we have determined rates of lipolysis, oxidation, and FA reesterification in healthy volunteers before and during IV infusion of a relatively large amount of TGs (~0.25 mmol · kg^{-1} · h^{-1}) with or without heparin under euglycemic-hyperinsulinemic conditions.

In the absence of heparin, only 29% of TGs infused over 4 hours (24.5 mmol) were lipolyzed. Addition of heparin resulted in virtually complete lipolysis (98%, or 84.8 mmol). This enhanced rate of lipolysis was reflected to some extent in higher plasma FFA concentrations, which increased to approximately 750 μmol · L^{-1} with heparin, remained at about the preinfusion level (~550 μmol · L^{-1}) without heparin, and decreased to approximately 50 μmol · L^{-1} without lipid infusion. In addition, the increased lipolysis of infused TGs caused plasma TG levels to be lower with heparin than without heparin (4.0 *v* 6.0 mmol · L^{-1}), although the difference was not statistically significant. Since in the presence of heparin essentially all of the infused TGs were lipolyzed, we assume that most of the TGs remaining in the plasma were newly synthesized, ie, were very–low-density lipoproteins (VLDL). To calculate TG-derived lipolysis, we have subtracted rates of lipolysis in controls (no lipid infusion) from those in subjects who received lipid. Fat infusion can produce insulin resistance[25] and thus may increase lipolysis. However, insulin concentrations in our studies were higher than needed to maximally suppress lipolysis.[26] Hence, lipid infusions per se were unlikely to influence lipolysis. In addition, we have assumed that endogenous VLDL production was similarly suppressed by insulin[27] during TG and saline infusions. We are not aware of data specifically addressing this question. It is therefore possible that we may have slightly overestimated lipolysis

from infused TGs if TG infusion increased insulin-suppressed VLDL production.

Fat oxidation has been shown in many studies to be closely correlated with plasma FFA concentration.[28-30] A major finding in this study was that FA oxidation rates were comparable despite significantly higher plasma FFA concentrations with than without heparin. The reason may have been that FA oxidation was already maximal with lipid infusion and could not be further increased with heparin despite an increase in FFA. In support of this contention, rates of fat oxidation have been reported by several laboratories to range from approximately 1.1 to approximately 3.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at plasma FFA concentrations ranging from approximately 400 to approximately 1,300 $\mu mol \cdot L^{-1}$.[26,29,31,32] On the other hand, our data also indicated that during TG infusions, 40% of the fat that was lipolyzed was oxidized, as compared with only 12% when TGs were infused together with heparin. As a result, there were fewer FFA available for reesterification.

Reesterification of FAs to TGs is the ultimate fate of FAs that are not oxidized. In this study, heparin increased rates of reesterification from infused TG almost fivefold (from 176 to 843 $\mu mol \cdot kg^{-1} \cdot 4\ h^{-1}$). Virtually all of this reesterification was "extracellular" or secondary reesterification (data not shown), in which FAs are transported in the blood to the liver, adipose tissue, and muscle, where they are reesterified.[33] Our results do not permit us to identify precisely the fate of reesterified FAs. Some reentered the circulation in the form of VLDL, and the remainder was probably deposited as storage fat in adipose tissue, muscle, and liver.

Lipolysis followed by reesterification can be considered a futile cycle in which energy is consumed without producing a net change in substrates. The energy cost of this TG/FA cycling can be estimated assuming that 1 mol FA recycled consumes 602 kJ.[19,20] We found that the caloric gain from oxidation of infused fat was approximately the same with and without heparin (293 *v* 276 $kJ \cdot 4\ h^{-1}$). Since the caloric cost of reesterification (126 *v* 33 $kJ \cdot 4\ h^{-1}$) was significantly higher with heparin, heparin actually decreased the net energy gain (167 *v* 243 $kJ \cdot 4\ h^{-1}$). The difference was not statistically significant, although it cannot be excluded that this was due to a type 2 statistical error.

There have been many reports of adverse effects associated with IV infusion of large amounts of artificial TG emulsions. For instance, the possibility of inhibition of reticuloendothelial system (RES) function in humans was suggested by the demonstration that IV infusion of large amounts of TG emulsions resulted in uptake of fat by hepatic macrophages (Kupffer cells),[34] depression of macrophage function,[35,36] inhibition of technetium-99 sulfur colloid clearance,[37] and increased mortality due to infectious complications in patients who preoperatively received 50% of their nonprotein calories in the form of lipid.[38] In addition, animal studies have shown that IV administration of TG emulsions reduced uptake of bacteria by liver and spleen.[39] Our finding of complete lipolysis of infused TGs with heparin suggested that much and perhaps all of the fat-induced impairment of RES function may be preventable by infusion of artificial TG emulsions together with heparin and insulin.

To our knowledge, data on rates of lipolysis, oxidation, and reesterification of infused artificial TG emulsions have not been reported previously. Others have determined rates of disappearance of fat particles from the circulation. Hallberg[4] differentiated exogenous from endogenous TGs by density-gradient separation and reported a half-life of disappearance of approximately 13 minutes after single injections of Intralipid (A.B. Vitrum, Stockholm, Sweden) in normal human subjects. Although these data were not directly comparable to our results, they nevertheless suggest that fat removal rates far exceed rates of lipolysis. This indicates that much of the infused artificial TG emulsions were removed without first being lipolyzed, ie, they were probably taken up by Kupffer cells and macrophages.

In summary, addition of heparin to IV infused LIPOSYN II and insulin greatly increased the amount of infused TG lipolyzed over 4 hours (to ~100% from ~30%) and increased reesterification of FAs (to ~80% from ~20%). Thus, heparin accelerated removal of artificial TGs from the blood and promoted their deposition into endogenous fat depots. It appears likely that this could reduce macrophage ingestion of exogenous fat and inhibition of RES function. On the other hand, heparin had no effect on the amount of TG oxidized. It also did not increase net energy production, although this may have been a consequence of the relatively small number of study subjects.

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