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The hypertriglyceridemic clamp technique. Studies using long-chain and structured triglyceride emulsions in healthy subjects

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Abstract

In a randomized crossover study, plasma kinetics of 2 different types of fat emulsions were studied in 8 healthy volunteers by using a hypertriglyceridemic clamp technique. The method involves the stabilization of serum triglyceride (TG) concentration during 180 minutes at a predetermined level (4 mmol/L) by adjustment of TG infusion rate by repeated online measurements of serum TG concentration. The fat emulsions under study were a long-chain fatty acid triglyceride (LCT) emulsion (Intralipid 20%, Fresenius Kabi, Sweden) and a structured triglyceride (STG) emulsion (Structolipid 20%, Fresenius Kabi) where medium- and long-chain fatty acids have been interesterified within a TG molecule. The hypertriglyceridemic clamp was found to have acceptable reproducibility when tested in 3 healthy individuals on 2 different occasions, as similar steady-state TG levels were obtained by infusing similar amounts of fat. The average (±SEM) TG concentration during the 180-minute clamp was similar for STGs and LCTs (4.0 ± 0.1 vs 3.9 ± 0.1 mmol/L; not significant), but the amount of fat that had to be infused was significantly higher during STG than during LCT clamping (0.31 ± 0.04 vs 0.21 ± 0.02 g TG per minute; $P < .05$). Higher serum levels of free fatty acids (1.80 ± 0.13 vs 0.96 ± 0.09 mmol/L; $P < .05$), free glycerol (1.30 ± 0.07 vs 0.76 ± 0.08 mmol/L; $P < .001$), and β-OH butyrate (1.61 ± 0.44 vs 1.17 ± 0.23 mmol/L; not significant) were obtained at the end of the clamp during infusion of STGs compared with LCTs. During infusion of STGs the medium-chain fatty acids octanoic (C:8) and decanoic acid (C:10) constituted approximately half of circulating fatty acids that correspond to the compositional ratio of the emulsion. Plasma lipoprotein lipase (LPL) concentration was higher during STG than during LCT clamping (6.06 ± 0.62 vs 3.15 ± 0.40 mU/mL; $P < .05$), and there was a positive correlation between the mean LPL concentration and the amount of infused TG during the steady-state period ($r = 0.58$; $P < .05$). In conclusion, the hypertriglyceridemic clamp method was found to give reproducible results and could be considered for comparison of metabolic clearance properties of different types of fat emulsions. The capacity of healthy subjects to eliminate STGs from blood was greater than for LCTs. An increased LPL activity induced by the higher TG infusion rate may have contributed to the increased metabolic clearance of STGs.

1. Introduction

Traditional fat emulsions for parenteral nutrition are based on soybean or safflower oils and contain almost entirely only long-chain fatty acids. During recent years, fat emulsions with different fatty acid compositions have been developed with the intention to further improve clinical effects during intravenous feeding. Fat emulsions blended with medium-chain fatty acid triglycerides (MCTs) and long-chain fatty acid triglycerides (LCTs) have been available for clinical use for several years. Other types of fat emulsions based on olive oil [1], fish oil [2], or structured triglycerides (STGs) [3,4] have been studied in clinical trials, and the potential merits of these emulsions have recently been reviewed [5,6].

A number of different techniques have been used to characterize the metabolism of intravenous fat emulsions. The intravenous fat tolerance test is one method that can be used for the study of plasma kinetics of artificial fat emulsions [7]. With this method, the plasma elimination rate is measured after an intravenous injection of a single bolus dose of fat. Other methods have also been developed that involve the use of a single constant infusion [8] or

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Table 1
Composition of the STG and LCT emulsions

Content/1000 mL	STG	LCT
Purified soybean oil (g)	–	200
Purified STGs (g)	200	–
Purified egg phospholipids (g)	12	12
Glycerol (USP) (g)	22.5	22.5
Water for injection (mL)	1000	1000
Energy content (MJ)	8.2	8.4
pH (approx.)	8	8
Mean molecular weight (approx.)	683	865
Proportion of- long to medium-chain fatty acids (%/%)		
By weight	64:36	100:0
On molar basis	50:50	100:0
Fatty acid composition (wt%)		
Caprylic acid (8:0)	27	–
Capric acid (10:0)	10	–
Palmitic acid (16:0)	7	13
Stearic acid (18:0)	3	4
Oleic acid (18:1)	13	22
Linoleic acid (18:2)	33	52
α-Linolenic acid (18:3)	5	8
Other	2	1
Total	100	100

3-stage constant infusion rates [9] or radioisotopes [10]. These methods have been used to study the effects of various physiologic or disease states on plasma elimination capacity of intravenous fat emulsions.

Conventional methods are, however, not suitable for comparisons among different types of fat emulsions for several reasons. First, the method for evaluation of plasma kinetics by nephelometry measures plasma fat particle concentration as light-scattering index [7]. The light-scattering index is, however, influenced by fat particle size and because this is a factor that varies between different fat emulsions, nephelometry cannot accurately be used to compare clearance properties of different types of emulsions. Second, there are differences in molecular weights between different fat emulsions due to differences in fatty acid composition. When infusing similar amounts of lipids by weight, more triglyceride (TG) molecules will be infused with a fat emulsion of lower molecular weight. As plasma or serum TG values by convention are presented in millimoles per liter, the reading value is influenced by the TG molar rather than TG weight infusion rate. Therefore, comparison among different fat emulsions should be undertaken with isomolar infusion rates.

An alternative approach to compare the metabolism of different types of fat emulsions is to use a hypertriglyceridemic clamp technique [11,12]. This method is based on the same principle as the glucose clamp technique [13] and involves the stabilization of TG concentration in blood at a predetermined level by adjustment of the TG infusion rate during repeated online measurements of TG concentration. The original description of the method involved a 5-hour lipid infusion with a predetermined TG level of 6 or 11 mmol/L [11].

In this study we modified the hypertriglyceridemic clamp method to involve the stabilization of plasma TG concentration at 4 mmol/L for 3 hours and compared the metabolic properties of an STG emulsion (Structolipid 20%, Fresenius Kabi, Sweden) and an LCT emulsion (Intralipid 20%, Fresenius Kabi) given to healthy volunteers.

2. Materials and methods

2.1. The hypertriglyceridemic clamp

The technique, as described by Carpentier et al [11], aims at keeping serum TG levels at 6 and 11 mmol/L during 4 hours and 1 hour, respectively. We have modified the technique so that serum TG concentration is kept as constant as possible at 4 mmol/L during 3 hours. Another difference, compared with the original description of the method, is the use of an automated apparatus (Reflotron, Boehringer Mannheim, Mannheim, Germany) for the enzymatic measurement of TG.

The subjects were studied after an overnight fast. Indwelling catheters were inserted into each arm, one for infusing fat emulsion and the other for obtaining blood samples. After a control period of about 30 minutes, basal blood samples were obtained. A primed constant infusion of fat emulsion at a rate of 0.013 g TG per minute per kilogram body weight was given for 20 minutes. After the priming period, the infusion rate of fat was reduced to about one fourth of the infusion rate used during the priming period and adjusted to maintain a TG level of 4 mmol/L as constant as possible. Our initial methodological studies showed that a steady-state TG level of 4 mmol/L uniformly could be obtained within 10 minutes. This level was kept as constant as possible during another 180 minutes by adjusting the fat infusion rate according to the TG values obtained from the Reflotron at the 10-minute measurements. However, the final presentations of the clamp results are based on the TG values analyzed after the clamp by a glycerol phosphate oxidase (GPO) method (see below).

When evaluating the data for an individual clamp, a deviation of no more than 6 of 18 measured TG values outside the interval of 3.5 to 4.5 mmol/L was accepted. The amount of infused fat (millimoles TG per liter) during the 180-minute steady-state hypertriglyceridemic period was considered to reflect TG clearance from the plasma compartment. The reproducibility of the hypertriglyceridemic clamp was tested in 3 healthy men. Intralipid 20% was used as substrate and 2 clamps were performed in each subject with a 4- to 7-month interval. Comparisons of steady-state TG levels and amounts of infused TG were made between the 2 tests of each subject.

2.2. Subjects

The study population comprised 8 healthy men with a median age of 32 years (range, 19-44 years) and a body mass index of 23 kg/m^2 (range, 19-25). Preinvestigational

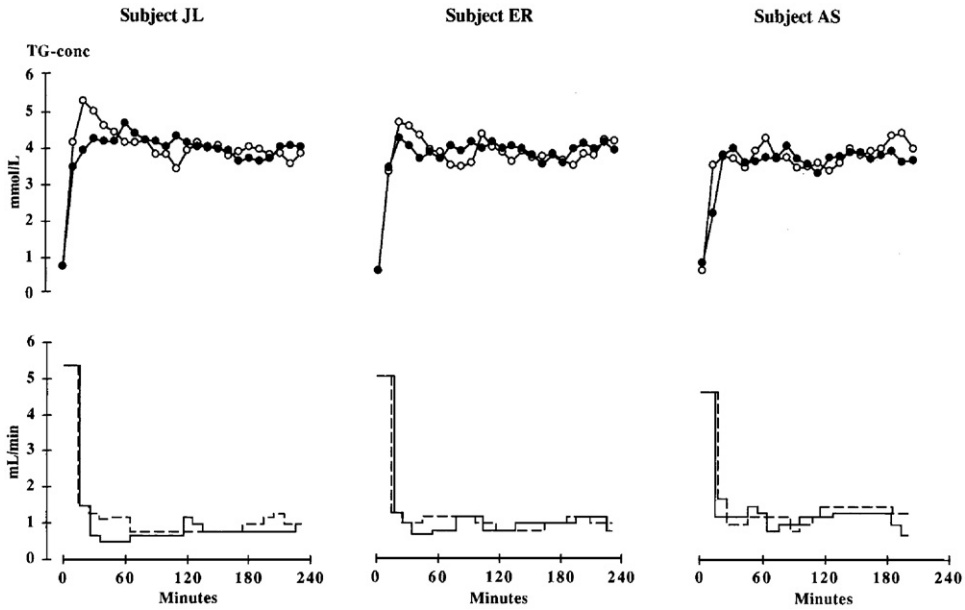

Fig. 1. The hypertriglyceridemic clamp performed in 3 healthy men (JL, ER, and AS) on 2 different occasions (4 to 7 months apart). TG concentrations obtained during the first and second study (top) are depicted by unfilled (○) and filled (●) symbols, respectively. Unbroken and broken lines for TG infusion rates denote the first and second study (bottom), respectively.

blood analyses and physical examinations had asserted that the subjects were healthy and, specifically, that there was no evidence of lipid metabolism abnormalities. None of the subjects were taking any medications. Before their participation, the nature, purpose, and potential risks of the study were explained, and their consent was obtained. The ethics committee at Karolinska University Hospital approved the experimental protocol.

2.3. Study design

The study was a comparative, randomized crossover study of plasma TG elimination during a hypertriglyceridemic clamp (as described above) with either an LCT emulsion (Intralipid 20%) or an STG emulsion (Structolipid 20%). The STG emulsion contains interesterified TGs from medium and long-chain fatty acids at a ratio of 36:64 wt% (50:50 mol%). The composition of STGs and LCTs are given in Table 1. A hypertriglyceridemic clamp using STGs as well as LCTs was performed in all subjects, with a time interval between tests of at least 7 days.

Blood samples were collected before and during the hypertriglyceridemic clamp for the determination of serum TG, total free fatty acid (FFA), and β-OH butyrate patterns, and plasma glucose, lipoprotein lipase (LPL), hepatic lipase (HL), and FFA patterns. Plasma volume was calculated from a regression equation based on height and weight and hematocrit [14].

At a preinvestigational visit at least 1 week before the study, lipolytic capacity was determined in each subject after an overnight fast. Blood samples were collected before and 15 minutes after infusion of heparin (10 IU heparin sodium per kilogram body weight) for analyses of plasma LPL and HL. Determination of lipolytic capacity was repeated in all subjects on the day after the last of the 2 clamp studies.

2.4. Analytical procedures

An automated enzymatic assay equipment (Reflotron) was used for rapid determination of serum TG concentration every 10 minutes during the clamp. The Reflotron provides a serum TG value in 190 seconds. Serum TG concentration was also measured by the routine method (an L-α-GPO method, Boehringer Mannheim) at the Department of Clinical Chemistry at our hospital. This method determines both free and TG-bound glycerol present in the samples [15]. Serum total FFA was measured by an enzymatic colorimetric test kit (Wako Chemicals, Nuess, Germany). The concentrations of free and esterified individual fatty acids were analyzed by reversed-phase high-performance liquid chromatography after derivation with fluorescent coumarin reagent. Quantification was performed by using standards with known concentrations of fatty acids [16].

Serum glycerol concentration was determined by an enzymatic kit (Boehringer Mannheim). Serum lipoprotein fractions, very low density lipoprotein (VLDL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), and their TG and cholesterol content was quantitated by a combination of ultracentrifugation and precipitation techniques [17,18]. Plasma LPL and HL activities were measured according to described techniques [19,20]. This assay uses antibodies to HL when determining LPL and 1 mol/L NaCl to suppress LPL in the assay of HL. Plasma glucose was analyzed by standard procedure with a Hitachi 705 apparatus.

2.5. Statistical analysis

Statistically significant differences were examined by the model for a 2-treatment, 2-period crossover design according to Hills and Armitage [21]. The treatment by period interaction, the period effect difference, and the treatment effect difference were tested. The normality assumption was checked with Shapiro-Wilk statistics. All data are presented as means ± SEM.

3. Results

The results of the reproducibility test whereby 3 healthy men were each tested on 2 occasions are shown in Fig 1. No significant differences in TG concentrations or TG infusion rates were noted in any of the subjects between the first and the second test.

The results of the hypertriglyceridemic clamps performed in 8 subjects are shown in Table 2 and Fig 2.

The mean serum TG values (measured with the GPO method) were, at all time points during the steady-state period (100 to 280 min), within the range of 3.5 to 4.5 mmol/L for both emulsions (Fig 2). The average TG concentrations during the steady-state period were similar for STGs and LCTs (4.0 ± 0.1 vs 3.9 ± 0.1 mmol/L, not significant [NS]).

The amount of fat that had to be infused to maintain a serum TG level of 4 mmol/L was significantly higher during STG than during LCT clamping (0.31 ± 0.04 vs 0.21 ± 0.02 g TG per minute; $P < .005$) (Table 2). On a molar basis, the difference between STG and LCT clamping was even more pronounced (0.45 vs 0.24 mmol TG per minute). In every subject, larger amounts had to be infused during STG than LCT clamping, with the difference ranging from ±8% to ±160% on a TG weight (gram) basis. A substantial part of the total amount of fat was infused during the priming period up to 100 minutes. The mean amount of emulsion used to obtain steady state at 100 minutes was 221 mL (44% of the total amount) for STGs and 172 mL (49% of total) for LCTs, a difference that was not statistically significant.

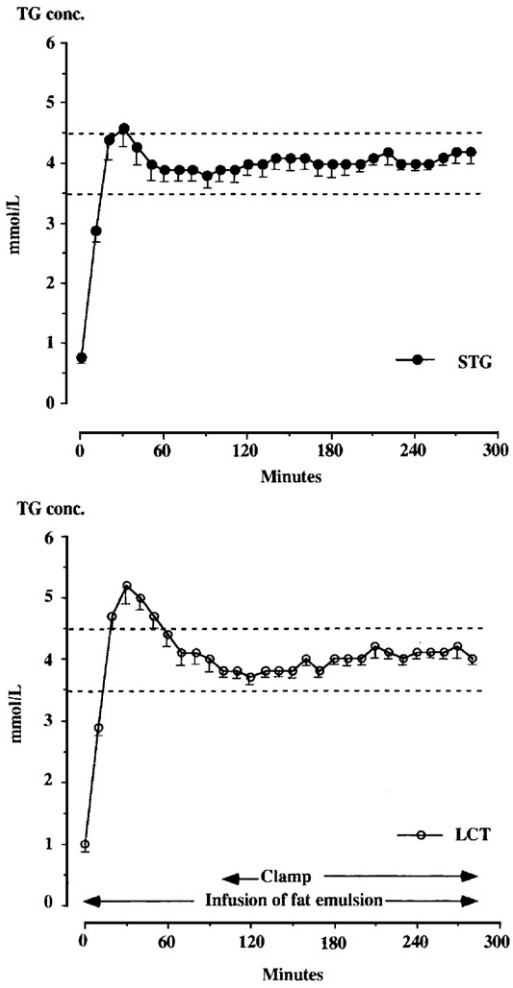

Fig. 2. TG concentration in 8 healthy subjects during a hypertriglyceridemic clamp with STGs (top) and LCTs (bottom). Mean values ± SEM.

There was no significant difference in serum TG values during clamping with STGs or LCTs measured either with the Reflotron or with the GPO method. There was

Table 2
Outcome of hypertriglyceridaemic clamps performed in 8 healthy individuals with either STGs or LCTs

Subject No.	Random order	Infused amount of TGs (g/mmol)				Plasma volume (L)		Steady state phase					
		Priming phase		Steady state phase				Mean TG concentration (mmol/L)		TG elimination rate (per liter plasma volume)			
										(g/min)		(mmol/min)	
		STG	LCT	STG	LCT	STG	LCT	STG	LCT	STG	LCT	STG	LCT
1	STG-LCT	42/209	33/166	67/334	56/281	2.89	3.04	4.2 ± 0.1	4.3 ± 0.1	0.13	0.10	0.64	0.51
2	LCT-STG	45/225	37/187	51/255	38/192	3.11	3.11	4.6 ± 0.0	4.2 ± 0.1	0.09	0.07	0.45	0.34
3	LCT-STG	45/226	33/166	48/240	35/173	2.84	2.69	4.6 ± 0.0	4.0 ± 0.0	0.09	0.07	0.47	0.36
4	STG-LCT	59/294	42/212	89/447	42/210	3.46	3.64	3.6 ± 0.0	3.6 ± 0.0	0.14	0.06	0.72	0.32
5	LCT-STG	24/122	25/126	27/133	24/119	2.58	2.49	4.0 ± 0.0	4.2 ± 0.0	0.06	0.05	0.29	0.26
6	STG-LCT	35/176	25/126	43/217	28/141	2.95	3.15	4.0 ± 0.1	3.7 ± 0.1	0.08	0.05	0.41	0.25
7	STG-LCT	64/322	48/238	66/332	50/248	3.60	3.67	3.7 ± 0.1	3.9 ± 0.1	0.10	0.07	0.51	0.37
8	LCT-STG	38/191	30/152	53/263	30/150	3.17	3.06	3.5 ± 0.1	3.6 ± 0.0	0.09	0.05	0.46	0.27
Mean		44/221	34/172	56/278	38/189	3.08	3.11	4.0	3.9	0.10	0.07	0.49	0.33
SEM		5/23	3/14	7/33	4/19	0.10	0.10	0.1	0.1	0.01	0.01	0.04	0.03

excellent correlation between these 2 methods for both LCTs (TG_{Ref} = 1.09TG_{GPO} − 0.331; n = 30; r^2 = 0.959) and STGs (TG_{Ref} = 1.05TG_{GPO} − 0.035; n = 30; r^2 = 0.958), indicating that the Reflotron is accurate enough for the determination of TG during the hypertriglyceridemic clamp.

There was a prompt and pronounced increase in the serum levels of total FFA during the priming phase (Table 3). At the end of the clamp, total FFA concentration was significantly higher with STGs than with LCTs (1.80 ± 0.13 vs 0.96 ± 0.09 mmol/L; $P < .05$). Average fatty acid pattern at the end of infusion is shown in Fig. 3. During infusion of STGs there was a marked increase in the concentrations of octanoic acid (C:8) and decanoic acid (C:10) concentrations and these constituted about half of the circulating fatty acids at the end of the clamp in this group. The levels of linoleic (C18:2, ω-6) and α-linolenic acid (C18:3, ω-3) increased during infusion of STGs and LCTs to approximately the same extent. Thus, the higher total fatty acid levels observed during STG infusion could almost entirely be accounted for by a higher concentration of medium-chain fatty acids (Fig. 3).

The concentration of free glycerol was significantly higher at the end of the clamp during STG than during LCT infusion (1.30 ± 0.07 vs 0.76 ± 0.08 mmol/L; $P < .001$) and the levels were stable during the entire steady-state TG period (Table 3). Serum free glycerol concentration during STG infusion was about 76% higher than during LCT infusion. Taking into account that the TG infusion rate (by weight) of STGs was approximately 50% higher, only a small fraction of the difference in free glycerol could be accounted for by increased TG hydrolysis during STG infusion.

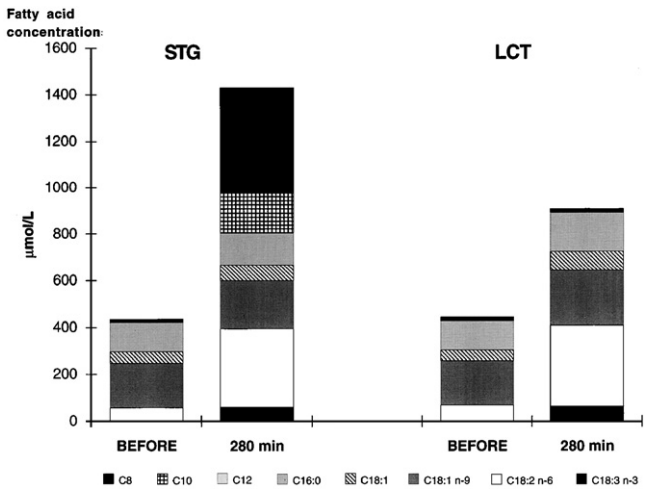

Fig. 3. Plasma fatty acid distribution in 8 healthy subjects before infusion and at the end of the steady-state period (280 minutes) during a hypertriglyceridemic clamp with STGs and LCTs.

Serum β-OH butyrate concentration at the end of the clamp was 6 to 8 times higher than that during basal conditions. Although β-OH butyrate levels tended to be higher during STG than LCT infusion, the difference did not reach statistical significance probably because of large interindividual differences. Plasma LPL levels increased in response to fat infusion and reached significantly higher levels during STG than LCT infusion (average during clamp, 5.4 ± 0.5 vs 3.2 ± 0.4 mU/mL; $P < .001$). LPL levels remained stable during LCT clamping but increased during the entire clamp in the STG group (Table 3). There was a positive correlation between mean plasma LPL

Table 3
Concentrations of substrates and LPL in 8 healthy men before and during a hypertriglyceridemic clamp with an STG or an LCT fat emulsion

	Baseline	Minutes in clamp			
		0	60	120	180
TG (mmol/L)					
STG	0.8 ± 0.1	3.9 ± 0.2	4.1 ± 0.2	4.1 ± 0.2	4.2 ± 0.2
LCT	1.0 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1
Total FFA (mmol/L)					
STG	0.38 ± 0.07	1.18 ± 0.09*			1.80 ± 0.13***
LCT	0.41 ± 0.08	0.87 ± 0.10			0.96 ± 0.09
Glycerol (mmol/L)					
STG	0.09 ± 0.02	1.35 ± 0.11***	1.33 ± 0.11***	1.30 ± 0.05***	1.30 ± 0.07***
LCT	0.11 ± 0.02	0.72 ± 0.07	0.72 ± 0.07	0.77 ± 0.06	0.76 ± 0.08
β-OH butyrate (mmol/L)					
STG	0.20 ± 0.07				1.61 ± 0.44
LCT	0.19 ± 0.07				1.17 ± 0.23
LPL (mU/mL)					
STG	1.19 ± 0.31		4.98 ± 0.55**	5.14 ± 0.53**	6.06 ± 0.62***
LCT	1.06 ± 0.22		3.29 ± 0.46	3.43 ± 0.39	3.15 ± 0.40
Glucose (mmol/L)					
STG	4.86 ± 0.09	4.93 ± 0.23	4.63 ± 0.10	4.54 ± 0.10	4.40 ± 0.06
LCT	4.88 ± 0.15	4.86 ± 0.13	4.76 ± 0.13	4.73 ± 0.10	4.69 ± 0.10

Values are mean ± SEM.
* $P < .05$.
** $P < .01$.
***$P < .001$, STG vs LCT.

Table 4
Concentration of total TGs and cholesterol and their fractions in LDL, HDL, and VLDL in 8 healthy men before and during hypertriglyceridemic clamp with an STG or an LCT fat emulsion

	Baseline	Minutes in clamp	
		120	180
TG (mmol/L)			
Total			
STG	0.75 ± 0.08	4.31 ± 0.15	4.44 ± 0.20
LCT	0.91 ± 0.08	4.15 ± 0.09	4.03 ± 0.09
LDL			
STG	0.26 ± 0.03	0.36 ± 0.02	0.34 ± 0.03
LCT	0.21 ± 0.02	0.31 ± 0.03	0.21 ± 0.02
HDL			
STG	0.11 ± 0.01	0.95 ± 0.07*	0.98 ± 0.07*
LCT	0.11 ± 0.01	0.49 ± 0.03	0.51 ± 0.03
VLDL			
STG	0.39 ± 0.07	3.05 ± 0.13	3.15 ± 0.17
LCT	0.53 ± 0.07	3.35 ± 0.10	3.28 ± 0.09
Cholesterol (mmol/L)			
Total			
STG	4.71 ± 0.18	4.80 ± 0.17	4.91 ± 0.16
LCT	4.74 ± 0.26	4.79 ± 0.26	4.78 ± 0.26
LDL			
STG	2.99 ± 0.17	2.88 ± 0.15	2.89 ± 0.16
LCT	3.09 ± 0.25	2.91 ± 0.22	2.90 ± 0.23
HDL			
STG	1.40 ± 0.08	1.28 ± 0.08	1.31 ± 0.09
LCT	1.34 ± 0.08	1.20 ± 0.10	1.19 ± 0.08
VLDL			
STG	0.30 ± 0.05	0.66 ± 0.05	0.70 ± 0.05
LCT	0.30 ± 0.04	0.68 ± 0.03	0.68 ± 0.04

Values are mean ± SEM.
* $P < .05$, STG vs LCT.

concentration and the amount of infused TG during the steady-state period ($r = 0.58$; $P < .05$). There was no significant change in lipolytic capacity (either for LPL or for HL) between the pre- and postinvestigational analysis, indicating that the hypertriglyceridemic clamp had not compromised lipolytic capacity: LPL concentration before vs after clamp, 202 ± 17 vs 225 ± 24 mU/mL (NS) and HL concentration before vs after clamp, 422 ± 28 vs 439 ± 29 mU/mL (NS). Plasma glucose levels did not change during the hypertriglyceridemic clamp either with STGs or with LCTs (Table 3).

There was an increased TG concentration in the VLDL and HDL fractions of both groups, and steady-state levels seemed to be present at the end of the infusion periods (Table 4). The increment in HDL TGs was more pronounced during STG than LCT infusion. The infusions had little effect on total cholesterol levels, but there was a tendency to increase in VLDL cholesterol and decrease in HDL cholesterol of both emulsions.

4. Discussion

The present study demonstrates that the Reflotron method is sufficiently fast as well as accurate for TG measurement when using the hypertriglyceridemic clamp. Furthermore, the clamp technique is considered to have sufficient reproducibility based on repeated clamp studies in the same individuals.

Almost twice as much molar TGs of STGs than LCTs had to be infused to obtain steady-state clamp levels. This indicates that the capacity of healthy subjects to clear STGs from plasma is significantly greater than for LCTs. An increased rate of TG hydrolysis during infusion of STGs was also evident from the higher free glycerol, total FFA, and β-OH butyrate levels observed during infusion.

With the hypertriglyceridemic clamp technique the TG elimination rate corresponds to the amount of TG infused during the steady-state period. The hypertriglyceridemic clamp technique for the evaluation of metabolic clearance of TG is based on the same principle as the glucose clamp technique for assessment of glucose metabolism. With both methods, the infusion rate of the substrate (TG or glucose) is a measure of its elimination rate from the vascular compartment during a specific period during which the substrate level has been kept constant. With the hypertriglyceridemic clamp method, steady-state TG concentration was in all cases obtained within 100 minutes after the start of the fat infusion. After the assigned TG concentration of 4 mmol/L had been achieved, only minor adjustments of the TG infusion rate were required to maintain steady-state TG levels. This is in contrast to the situation during glucose clamping where the glucose infusion rate under physiologic conditions has to be increased because of an increased insulin response.

Compared with the original description of the hypertriglyceridemic clamp method [11], several modifications were made because the method was initially designed for the study of the changes in lipoprotein patterns over a relatively long infusion period (5 hours) and high TG steady-state levels (6 and 11 mmol/L) were used. Furthermore, a combined infusion of glucose and amino acids was administered to avoid endogenous metabolic changes that would occur during a relatively long study period. The aim of the present study was different from that stated in the original description. Our objective was to study the metabolic clearance of different types of fat emulsions, and a TG clamp level of 4 mmol/L was chosen. This TG level was chosen based on clinical experience from TG values measured during infusion of fat emulsions. The mean dose of LCT fat given during the approximately 4.5-hour-long study was 360 mL, which would be a relevant daily dose under clinical conditions.

In a study by Iriyama et al [12], the authors clamped their subjects for 160 minutes at 3 different TG levels (2, 3-4, and 4-5 mmol/L). They found that after the priming period had been completed, similar TG infusion rates (approximately 0.12 g TG per kilogram per hour) rendered steady-state conditions at all 3 TG levels. This indicates first-order kinetics within a TG substrate concentration of 2 to 5 mmol/L. The plasma elimination rate of Intralipid 20% was 0.16 g TG per kilogram per hour in the present study,

which was 33% higher than that found by Iriyama et al in their study with an identical fat emulsion. The underlying mechanism behind this difference is unknown but may relate to differences in the metabolic handling of intravenous fat between the Scandinavian and Japanese population.

The concentration of free glycerol followed the same pattern as the TG infusion rate with a more accentuated increase during STG infusion. Both emulsions contain the same amount of free glycerol, and the concentration could therefore be expected to increase with an increased TG infusion rate. The standard TG assay (GPO method) as well as the Reflotron method determines both free and bound glycerol and is therefore associated with a systematic error when measuring TG during infusion of fat emulsions containing glycerol. Both methods, therefore, overestimate the true TG concentration in the samples. The observed difference in serum free glycerol at the end of the clamps was in the range of 0.5 to 0.6 mmol/L (Table 3). Higher infusion rates would increase free glycerol concentration and, thus, underestimate the true TG concentration during a steady-state situation. The higher elimination rate observed for the STGs thus cannot be accounted for by measurement of free glycerol when determining TG concentration.

There was a rapid increase in total FFA concentration during the priming period (Table 3). Total FFA concentration tended to further increase in subjects receiving STGs. The increase in long-chain fatty acids was similar during clamping with STGs and LCTs (Fig. 3). The concentration of decanoic acid (C:10) and in particular the concentration of octanoic acid (C:8) increased markedly during STG infusion, and these medium-chain fatty acids were responsible for the higher total fatty acid levels observed with infusion of STGs in relation to LCTs (Table 3).

The STG emulsion contains 50 mol% less long-chain fatty acids than the LCT emulsion, and the TG infusion rate on a molar basis was 86% higher during STG clamping. The similar long-chain fatty acid levels during infusion of the different emulsions could therefore be expected. At the end of the clamping period, the molar proportion of medium- to long-chain fatty acid concentration in plasma during STG infusion was similar to the compositional ratio of the emulsion. This indicates that there was no apparent preferential hydrolysis of medium- over long-chain fatty acids as has been shown to be the case with MCT emulsions [22,23]. In this respect, STG emulsions may be advantageous compared with emulsions blended with MCTs and LCTs.

LPL concentration increased during clamping with both fat emulsions, but the increase was more pronounced with STG emulsion. We have previously observed a direct relationship between LPL activity and TG levels during infusion of an MCT/LCT and pure LCT emulsion [24]. In the present study, we found a positive correlation between mean LPL concentration and the amount of infused TG. The exact mechanism behind the increased LPL levels during fat infusion is not clear. Peterson et al [19] have suggested that the accumulation of fatty acids at endothelial sites of lipolysis, rather than the TGs as such, displaces LPL into the circulating blood. An alternative explanation could be that the STG itself somehow could be a greater stimulant for LPL release than the LCTs. In the present study, the concentration of medium-chain fatty acids (and total FFAs) reached higher levels during STG rather than LCT infusion. The design of the present study does not permit elaboration as to the relative contribution of fatty acids or TGs to the increased LPL levels. As suggested by our previous studies [19], the present study demonstrates a reciprocal interaction between TG and LPL turnover. LPL activity is dependent on the TG infusion rate, and increased LPL activity will have a plasma TG lowering effect.

We conclude that the hypertriglyceridemic clamp technique was found to be reproducible and useful for the comparison of metabolic clearance of STGs and LCTs. Whether the technique can be used to characterize TG metabolism in different physiologic or pathologic conditions requires further study.

References

[1] Goulet O, de Potter S, Antebi H, Driss F, Colomb V, Bereziat G, et al. Long-term efficacy and safety of a new olive oil–based intravenous fat emulsion in pediatric patients: a double-blind randomized study. Am J Clin Nutr 1999;70:338-45.

[2] Tsekos E, Reuter C, Stehle P, Boeden G. Perioperative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcome after major abdominal surgery. Clin Nutr 2004; 23:325-30.

[3] Sandström R, Hyltander A, Körner U, Lundholm K. Structured triglycerides to postoperative patients. A safety and tolerance study. JPEN J Parenter Enteral Nutr 1993;17:153-7.

[4] Nordenström J, Thörne A, Olivecrona T. Metabolic effects of infusion of a structured triglyceride emulsion in healthy subjects. Nutrition 1995;11:269-74.

[5] Deckelbaum RJ, Calder P, Carpentier YA. Using different intravenous lipids: underutilized therapeutic approaches? Curr Opin Clin Nutr Metab Care 2004;7:113-7.

[6] Hasselmann M, Reimund J-M. Lipids in the nutritional support of the critically ill patients. Curr Opin Crit Care 2004;10:449-55.

[7] Carlson LA, Rössner S. A metabolic study of an intravenous fat tolerance test with Intralipid emulsion. Scand J Clin Lab Invest 1972;29:217-80.

[8] Hallberg D. Studies on the elimination of exogenous lipids from blood stream. The kinetics of the elimination of a fat emulsion studied by a constant infusion technique in man. Acta Physiol Scand 1965; 64:299-305.

[9] Robin AP, Nordenström J, Askanazi J, Elwyn DH, Carpentier YS, Kinney JM. Plasma clearance of fat emulsion in trauma and sepsis. Use of a three-stage lipid clearance test. JPEN J Parenter Enteral Nutr 1980;4:505-10.

[10] Nordenström J, Carpentier YA, Askanazi J, Robin AP, Elwyn DH, Hensle TW, et al. Metabolic utilization of intravenous fat emulsion during total parenteral nutrition. Ann Surg 1982;196:221-31.

[11] Carpentier YA, Kinney JM, Siderova VS, Richelle M, Nishiwaki H, Olivecrona T, et al. Hypertriglyceridaemic clamp: a new model for studying lipid metabolism. Clin Nutr 1990;9(Suppl):1 [abstract].

[12] Iriyama K, Tsuchibashi T, Miki C, Kalembeyi I, Li H, Urata H, et al. Elimination rate of fat emulsion particles from plasma in Japanese subjects as determined by triglyceride clamp technique. Nutrition 1996;12:79-82.

[13] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique. A method for quantifying insulin secretion resistance. Am J Physiol 1979;237:E214-23.
[14] Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962;51:224-32.
[15] Wahlefeld AW. Triglycerides. Determination after enzymatic hydrolysis. In: Bergmeyer HV, editor. Methods of enzymatic analysis, vol. 4. 2nd ed. Verlag Chemie Weinheim. Academic Press, Inc. New York: 1974. p. 1831-5.
[16] Tsuchiya H, Hayashi T, Sto M, Tatsumi M, Takagi N. Simultaneous separation and sensitive determination of free fatty acids in blood plasma by high-performance liquid chromatography. J Chromatogr 1984;309:43-52.
[17] Carlson K. Lipoprotein fractionation. J Clin Pathol 1973;26:32-7.
[18] Lopez-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882-4.
[19] Peterson J, Olivecrona T, Bengtsson-Olivecrona G. Distribution of lipoprotein lipase and hepatic lipase between plasma and tissues; effect of hypertriglyceridemia. Biochim Biophys Acta 1985;837: 262-70.
[20] Peterson J, Bihain BE, Bengtsson-Olivecrona G, Deckelbaum RJ, Carpentier YA, Olivecrona T. Fatty acid control of lipoprotein lipase. A link between energy metabolism and lipid transport. Proc Natl Acad Sci U S A 1990;87:909-13.
[21] Hills M, Armitage P. The two-period crossover clinical trial. Br J Clin Pharmacol 1979;8:7-20.
[22] Deckelbaum RJ, Hamilton J, Moser A, Bengtsson-Olivecrona G, Butbul E, Carpentier Y, et al. Medium-chain vs. long-chain triacylglycerol emulsion hydrolysis by lipoprotein lipase and hepatic lipase: implications for the mechanism of lipase action. Biochemistry 1990;29:1136-42.
[23] Lutz O, Lave T, Frey A, Meraihi Z, Bach AC. Activities of lipoprotein lipase and hepatic lipase on long- and medium-chain triglyceride emulsions used in parenteral nutrition. Metabolism 1989;38:507-13.
[24] Nordenström J, Neeser G, Olivecrona T, Wahren J. Effect of medium- and long-chain triglyceride infusion on lipoprotein and hepatic lipase in healthy subjects. Eur J Clin Invest 1991;21:580-5.