

Original article

Particle size determines effects of lipoprotein lipase on the catabolism of n-3 triglyceride-rich particles



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SUMMARY

Background & aims: The catabolic pathways of n-3 triglyceride (TG) rich particles (n-3 TGRP) have not been clearly elucidated. In this study, we investigated the effects of lipoprotein lipase (LPL) on the catabolism of n-3 TGRP compared to n-6 TGRP in vivo and in vitro, and we determined whether particle size affects the biological functions of LPL in n-3 TGRP catabolism.

Methods: Four types of lipid emulsions, chylomicron (CM)-sized n-3 TG and n-6 TG emulsions, and very low density lipoprotein (VLDL)-sized n-3 TG and n-6 TG emulsions, were labeled with 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and administered via a bolus injection to LPL gene knockout (LPL^{−/−}) mice in vivo and were added to cultured LPL miRNA-transfected 3T3-L1 adipocytes in vitro.

Results: With CM-sized emulsions, a reduction in LPL expression in LPL^{−/−} mice had almost no effect on tissue uptake of n-3 TG emulsions with smaller changes in their initial blood clearance; however, greater effects were observed for VLDL-sized n-3 TG emulsions with respect to tissue uptake with greater changes in their initial blood clearance, compared to n-6 TG emulsions with the same size. In vitro, LPL miRNA transfection had smaller effects on CM-sized and greater effects on VLDL-sized n-3 TG emulsions, with respect to particle uptake, cell TG mass, particle-cell binding and particle lipolysis.

Conclusion: These results suggested that LPL is more important for catabolism of n-3 TGRP of smaller size; whereas it is essential for catabolism of all sizes of n-6 TGRP.

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1. Introduction

N-3 fatty acids have “beneficial” biological activities with respect to lipid metabolism, blood pressure, vascular function, cardiac rhythms, platelet function, inflammatory responses, and retinal and brain function, as well as the prevention of some chronic diseases [1–5]. Recently, n-3 triglycerides (TG) have been introduced into lipid emulsions for clinical nutritional support [6,7]. Traditionally, TGRP in blood were believed to be initially hydrolyzed by lipoprotein lipase (LPL) on the surface of endothelial cells, with the remnants being transported to the liver for catabolism via several

cellular receptors [8,9]. However, more recently, it has been confirmed that large-size TGRP can be cleared as intact whole particles by different tissues [10,11] and that many factors, including TG composition and particle size, may affect their catabolism [12,13]. Previously, we reported that n-3 TG-containing emulsions were removed from blood faster in mouse models by different pathways compared with soy oil (n-6 TG) emulsions [13–15]. The removal of intravenous (CM-sized) n-6 TG emulsions was modulated by apolipoprotein E (apoE), the LDL receptor (LDL-R), and lactoferrin-sensitive pathways in vivo and in vitro, whereas the clearance of intravenous n-3 TG emulsions was independent of these pathways [14,15]. To determine the role of lipoprotein lipase (LPL), we intravenously injected heparin and Triton WR 1339 to interfere with LPL function in mice and found that the tissue targeting of n-3 TG emulsion particles mediated by LPL is different from that of n-6 TG emulsions, particularly in the adipose tissue, and this difference was affected by particle size [13,14]. VLDL-sized n-3 TG emulsion particles were taken up much more by adipose tissue and this adipose

Abbreviations: CM, chylomicron; FO, fish oil; LPL, lipoprotein lipase; TGRPs, triglyceride rich particles; VLDL, very low density lipoprotein; WT, wide type.

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tissue uptake was substantially reduced after heparin injection [13], suggesting a key role of LPL in catabolism of n-3 TGRP with smaller size. Thus, in this study, LPL+/- mice and LPL miRNA-transfected adipocytes were used to further investigate effects of the reduced LPL expression on the catabolism of n-3 TGRP in vivo and in vitro, as well as to determine whether particle size affects the biological functions of LPL in n-3 TGRP catabolism.

2. Materials and methods

2.1. Lipid emulsions

Two types of intravenous lipid emulsions (10 g TG/100 mL) rich in n-6 TG (soybean oil) or n-3 TG (fish oil), were obtained from Sino-Swed Pharmaceutical Corp. Ltd. These emulsions were similar in particle size and homogeneity with mean diameters at 280 nm, similar to more heterogeneous chylomicrons (CM), which average between 250 and 400 nm in diameter. To determine catabolism of the emulsions, we labeled them with the fluorescent probe 1,1'-diiododecyl-3,3,3',3'-tetramethylindolyl-carbocyanine perchlorate (Dil), since lipoproteins labeled with this probe have been shown to have the same chemical composition and physical properties as native lipoproteins, and low nonspecific bindings. Also the Dil is firmly bound to the lipoproteins with little exchange with other lipoproteins. The labeling was conducted according to the methods modified [16,17]. In brief, the two emulsions were labeled with Dil (catalog number 897933, Invitrogen Biotechnology Co. Ltd.) by incubating the mixture of 10 µg Dil and 20 mg TG at 37 °C for 6 h. After incubation, the free Dil was removed by centrifugation (12,000 rpm, 10 min) three times, and the resulting emulsions were stored at 4 °C and used for further experiments within 7 days.

As well, two types of smaller-sized very low density lipoprotein (VLDL)-sized emulsions, n-6 TG (soybean oil) and n-3 TG (fish oil), were produced as previously described [15]. Briefly, TG (80 mg) (Aladdin Chemistry Co. Ltd. Shanghai) was mixed with a 4:1 weight ratio of egg yolk phosphatidylcholine (Avanti Polar Lipids, Inc.). After the emulsions were fully evaporated under N₂, the dried lipids were re-suspended in buffer (150 mM NaCl and 0.24 mM EDTA, pH 8.4, density 1.006 g/mL) at 60 °C with the addition of sucrose (1 g/10 mL) and sonicated for 1 h at 42 °C at 140 W under a stream of N₂ using a Branson Sonifier model 450 (Branson Scientific, Melville, NY). After sonication, 1 mg Dil was added to the emulsion solution and sonicated at 16 W for 1 min. Next, the solution was dialyzed for 24 h to remove the sucrose and centrifuged for 30 min at 45,000 rpm three times in a Beckman Type 90 Ti swinging bucket rotor to remove the free Dil and dense phospholipid-rich liposomes with trapped sucrose. The emulsions in the top layer were collected and used for the animal and cell experiments.

To determine emulsion particle size distribution, the mass ratios of TG to phospholipid were analyzed, which is correlated closely with particle size. The TG content was assayed using an enzymatic procedure with the TG GPO-PAP kit (catalog number CH0104151, Sichuan MAKER Biotechnology Co. Ltd.), and phospholipids were measured according to the Bartlett procedure [18]. Emulsions with different TG compositions showed very similar particle size distribution in either CM size or VLDL size. The ratios measured were 12.98 ± 1.6 and 12.87 ± 1.4 for CM-sized n-3 TG and n-6 TG emulsions, respectively, which were consistent with that for CM size (diameter 250–300 nm). VLDL-sized n-6 TG and n-3 TG emulsions had TG/phospholipid ratios of 5.0 ± 0.9 and 4.9 ± 1.0 respectively, which were consistent with that for VLDL size (diameter 50–100 nm). Furthermore, examination by laser light scattering photometry (DynaPro NanoStar, Model no. WDPN-06, Wyatt Technology Corporation, USA) indicated 301 ± 23 nm and 310 ± 21 nm in diameter with CM-sized n-3 TG and n-6 TG

emulsions, and 99 ± 6 nm and 100 ± 6 nm with VLDL-sized n-3 TG and n-6 TG emulsions, respectively.

The relative fatty acid composition (by weight) of emulsion TG determined by gas–liquid chromatography was shown in Table 1. CM-sized or VLDL-sized emulsions with the same TG compositions had very similar fatty acid profiles.

2.2. Animals

LPL+/- C57BL/6J mice were purchased from and housed at standard rooms in the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, Peking Union Medical College (CAMS&PUMC). Stock LPL+/- C57BL/6J mice were mated to breed pups preparing for experiments. Genotyping was done using genomic DNA extracted from tails by PCR and was designated LPL-/-, LPL+/- and LPL+/+ (WT) accordingly [19,20]. We used LPL+/- pups because LPL-/- pups live a very short time after birth. After weaning, the LPL+/- pups were kept on a normal chow diet containing 4.5% fat and water ad libitum and were sacrificed for analysis at 12–15 weeks old. The WT C57BL/6J mice of the same age were used as controls. All of the mice were provided with standard pellet rodent diets and water ad libitum. Before the following experiments, the LPL mRNA expression in mouse tissue and plasma LPL activity were measured (See procedures in Support information).

The mice were anesthetized via an intraperitoneal injection of Avertin (125 mg/kg). Each mouse was administered 50 µL of emulsion solution (diluted with 0.9% NaCl) containing 2 mg of TG by a bolus injection via the femoral vein. Emulsion clearance in the blood was assessed by measuring the fluorescence intensity of Dil in retro-orbital blood (50 µL), which was drawn at 0.5, 2, 5, 10, and 25 min by heparinized capillary tubes following an emulsion injection. The mice were sacrificed immediately by decapitation and perfused with 0.9% NaCl-containing heparin (2 units/mL). The organs and tissues (heart, lung, liver, epididymal fat and muscle) were dissected free of the surrounding tissue and transferred to a -80 °C freezer until further analysis. All of the animal experiments were performed from 0800 h to 1200 h in compliance with the guidelines of the Animal Care and Use Committee of the Institute of Laboratory Animal Sciences, CAMS&PUMC.

Blood and tissues were homogenized using a homogenizer, and a chloroform/methanol mixture (2/1, v/v) was used to extract lipids from the tissue homogenates [14,15]. The fluorescence intensity of Dil in the extracted lipids was measured using an ELISA microplate reader (SAFIRE II-Basic, Tecan, USA). The fluorescence intensity in blood was expressed as the percentage of the injected dose remaining in the whole blood. Tissue uptake was expressed as the

Table 1
The relative fatty acid composition (by weight) of emulsion TG.

Fatty acids	CM size		VLDL size	
	n-6 TG emulsion	n-3 TG emulsion	n-6 TG emulsion	n-3 TG emulsion
C14:0	0.1%	—	0.1%	—
C16:0	11.7%	20.9%	11.5%	16.0%
C16:1	0.1%	—	0.1%	—
C18:0	4.9%	5.1%	4.3%	4.1%
C18:1	20.8%	17.4%	21.7%	20.8%
C18:2(n-6)	54.9%	0.3%	54.6%	0.1%
C18:3(n-6)	—	0.2%	—	0.1%
C18:3(n-3)	6.9%	5.4%	7.4%	7.3%
C20:3(n-6)	—	2.2%	—	2.1%
C20:4(n-6)	0.2%	0.1%	—	0.1%
C20:4(n-3)	—	2.3%	—	2.5%
C20:5(EPA,n-3)	—	22.9%	—	27.2%
C22:4(n-6)	—	3.2%	—	2.7%
C22:6(DHA,n-3)	—	18.6%	—	15.5%

percentage of the recovered injected dose per organ, and the total adipose tissue and muscle mass were calculated as 15% and 42% of body weight, respectively [13]. The recovered injected dose was the sum of the fluorescence intensity from all organs and tissues assayed, including blood, at the end of experiments. Values were expressed as the mean \pm SD.

2.3. Cells

3T3-L1 preadipocytes were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% calf serum (CS), containing 100 U/mL penicillin, 100 μ g/mL streptomycin ($1 \times$ P/S) and L-glutamine (292 μ g/mL) (Peking Union Cell Center, China) at 37 °C in an atmosphere containing 5% CO₂ as described elsewhere [21]. To induce 3T3-L1 preadipocyte differentiation, cells were seeded in 20 mm dishes (1 mL medium/dish) at a density of 1×10^5 cells/dish to complete confluence for 2 days. Then, cell differentiation was induced by changing the medium to DMEM containing 10% fetal bovine serum (FBS), $1 \times$ P/S, 1 μ mol/L dexamethasone, 0.5 mmol/L 3-isobutyl-1-methylxanthine, and 5 μ g/mL insulin. After 48 h, the medium was changed to DMEM containing 10% FBS, $1 \times$ P/S, 5 μ g/mL insulin for 48 h. Thereafter, the medium was changed to DMEM containing 10% FBS and $1 \times$ P/S.

To inhibit the LPL expression, the microRNA (miRNA) expression vector plasmid, pcDNATM6.2-GW/EmGFPmiR, which was directed toward mouse LPL, was designed and synthesized by Invitrogen Inc. (Shanghai, China). Four pairs of oligomeric single-stranded DNA for

the miRNA of mouse LPL and one pair for the negative control were synthesized. The 5 double-stranded DNA segments, including a negative control, were each inserted into a plasmid pcDNATM6.2-GW/EmGFPmiR vector. After screening, the 4th pair of oligomeric single-strand DNA was found to have the most robust effects in reducing LPL mRNA expression (55% reduction) compared to the negative control (Support information). The differentiated adipocytes were transfected for 36 h with plasmid DNA using LipofectamineTM 2000 transfection reagents (Invitrogen Inc., USA) according to the manufacturer's specific protocol. The plasmid was internalized into the cells, resulting in a green fluorescence, as assessed using fluorescence microscopy, and the mRNA analysis showed that miRNA transfection reduced the LPL mRNA expression by 55% in adipocytes (the PCR conditions in Support information).

After transfection for 36 h, the cells were incubated for an additional 12 h in DMEM culture medium containing 10% lipoprotein-deficient serum (LPDS). Next, the cells were incubated with the emulsions (250 μ g of TG/mL) in DMEM containing 1% bovine serum albumin (BSA) for 4 h at 37 °C. After incubation, the cell media was removed and analyzed for non-esterified fatty acids (NEFAs) using a NEFA C kit (catalog number 294-63601, Wako Pure Chemical), and the cells were washed three times with ice-cold PBS, and then subsequently incubated for 1 h at 4 °C with PBS containing heparin (10 U/mL). The heparin solution was collected for the emulsion binding assay using fluorescence counts. The cells were used to determine the TG uptake by measuring the cell-associated fluorescence. We performed the TG mass assay using the TG GPO-

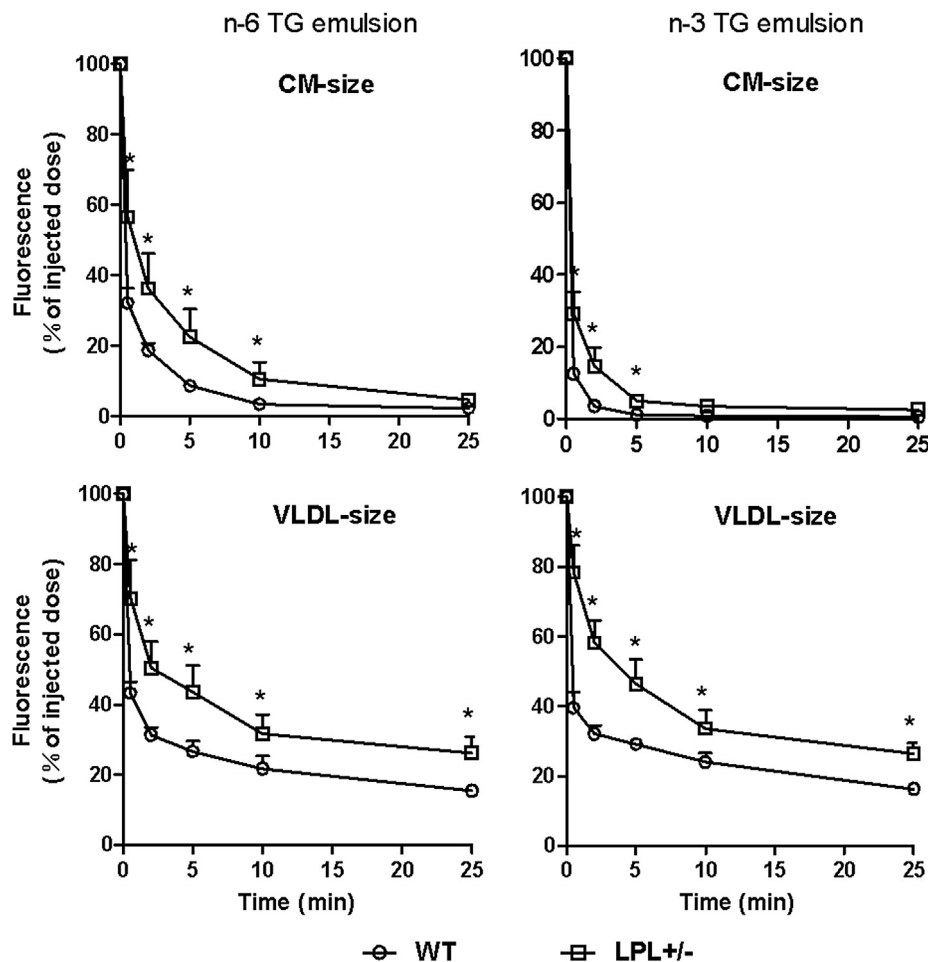


Fig. 1. Blood clearance of lipid emulsions with n-6 and n-3 TG in LPL^{+/−} mice: Dil-labeled emulsions (2 mg of TG/mouse) with different TG and particle size were intravenously injected into LPL^{+/−} and WT mice as indicated in Materials and Methods. The values represent the mean \pm SD ($n = 5-7$ in each group).

PAP kit for lipid extraction with hexane/isopropanol (3:2) as previously described [22]. In addition, emulsion particle uptake by cells was visualized under fluorescence microscopy. Oil red staining was performed to visualize the TG changes inside the cell.

The cell TG mass was defined as the cell TG uptake plus cell TG synthesis and baseline cell TG content (control values in the absence of emulsions) minus cell TG utilization. The baseline cell TG content was constant for all the experiments. In fact, the resulting NEFAs from the TGRPs hydrolyzed by LPL contributed little to the TG accumulation in the cells, because the NEFAs generated by LPL activity were trapped by the BSA medium and were prevented from entering the cell rapidly and/or were released back into the medium after uptake TG [23]. Thus, cell TG utilization was reflected by the changes in cell TG mass and uptake. In this study, the ratio of cell TG mass to uptake was used to determine cell TG utilization [22,23].

2.4. Statistical analysis

Student's *t*-tests were performed to evaluate the differences between the mean values of two groups (LPL+/- mice vs. WT mice, transfected cells vs. control cells, change in n-3 TG emulsions vs. n-6 TG emulsions after reduction of LPL expression). The results were expressed as the mean \pm SD. Significant differences were determined at $P < 0.05$.

3. Results

3.1. Determination of biochemical parameters, LPL expression and activity in LPL+/- mice

Plasma TG concentrations in LPL+/- mice (3.49 ± 1.67 mmol/L) were higher compared to WT mice (1.78 ± 0.21 mmol/L) ($n = 6$ in

each group) ($P < 0.05$). However, the plasma NEFA concentrations in LPL+/- mice (0.57 ± 0.28 mEq/L) were lower compared to WT mice (0.89 ± 0.12 mEq/L) ($P < 0.05$). Furthermore, LPL mRNA expression in the heart, lung, fat and muscle was reduced by 38%, 21%, 36% and 38%, respectively, in the LPL+/- mice compared to WT mice. Plasma LPL activity in LPL+/- mice (5.79 ± 1.12 $\mu\text{mol/h}^{-1}$ mL) was significantly lower than that (9.44 ± 2.12 $\mu\text{mol/h}^{-1}$ mL) in WT mice ($P < 0.05$).

3.2. Changes in the blood clearance of n-3 TG versus n-6 TG emulsions in LPL+/- mice

Following intravenous injection, all four different types of emulsions were cleared slower in LPL+/- mice compared to WT mice at the different time points ($P < 0.05$). However, changes in blood clearance between WT and LPL+/- mice were smaller for the CM-sized n-3 TG emulsions and greater for the VLDL-sized n-3 TG emulsions during the first 5 min, compared with n-6 TG emulsions with particles of the same size (Fig. 1).

3.3. Changes in tissue uptake of n-3 TG versus n-6 TG emulsions in LPL+/- mice

Fig. 2 shows reduced uptakes of CM-sized n-6 TG emulsion particles by the heart and adipose tissue in LPL+/- mice, compared to WT mice, and increased uptakes in the liver ($P < 0.05$); in contrast, n-3 TG emulsion particles targeting to the heart, adipose tissue and liver did not change ($P > 0.05$). With respect to VLDL-sized particles, both n-6 and n-3 TG emulsion uptakes in LPL+/- mice were reduced in the heart, adipose tissue and muscle, and increased in the liver, but changes in uptake of the n-3 TG emulsions by all these tissues were greater than for the n-6 TG emulsions ($P < 0.05$).

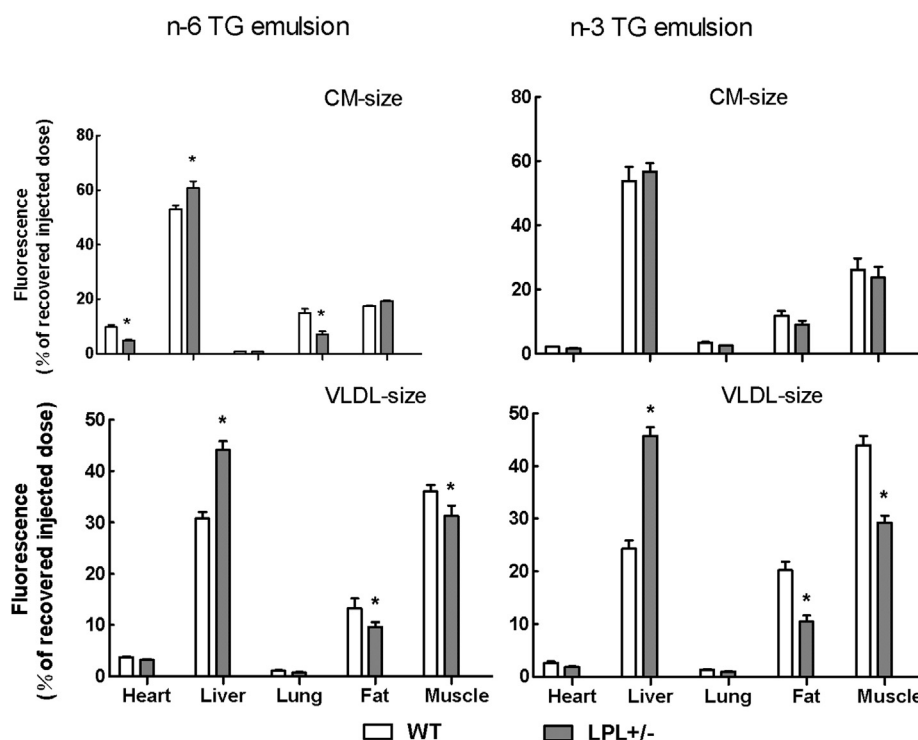


Fig. 2. Tissue uptake of lipid emulsions with n-6 and n-3 TG in LPL+/- mice: Dil-labeled emulsions (2 mg of TG/mouse) with different TG and particle size were intravenously injected into LPL+/- and WT mice. The fluorescence intensity in the tissues at 25 min after injection was measured as described in Materials and Methods. The values represent the mean \pm SD ($n = 5-7$ in each group). * indicates a significant difference ($P < 0.05$) compared to WT mice.

3.4. Effects of LPL on the catabolism of CM-sized n-3 versus n-6 TG emulsions in adipocytes

The differences in particle uptake, cell TG mass, particle-cell binding and TG lipolysis were examined in LPL miRNA-transfected 3T3-L1 adipocytes between n-3 TG and n-6 TG emulsions with CM-sized particles. As shown in Fig. 3, LPL miRNA transfection decreased particle uptake, particle-cell binding, cell TG mass and NEFAs in the medium with n-3 TG emulsions, and the changes in reduction were smaller with n-6 TG emulsions as compared ($P < 0.05$). The ratios of TG mass to uptake did not change in either the n-3 TG or n-6 TG emulsions when the cells were transfected with LPL miRNA ($P > 0.05$), indicating that cell TG utilization was not affected by LPL with CM-sized particle emulsions (Fig. 3).

3.5. Effects of LPL on catabolism of VLDL-sized n-3 TG versus n-6 TG emulsions in adipocytes

As shown in Fig. 4, in contrast to CM-sized emulsions, LPL miRNA transfection decreased particle uptake, particle-cell binding, cell TG mass and FFA release in the medium with VLDL-sized n-3 TG emulsions, and these changes were greater compared to values with VLDL-sized n-6 TG emulsions ($P < 0.05$). The ratios of

TG mass to uptake did not change in either the n-3 TG or n-6 TG emulsions when the cells were transfected with LPL miRNA ($P > 0.05$), indicating that cellular TG utilization was not affected by LPL with VLDL-sized particle emulsions.

4. Discussion

The catabolism of lipid emulsions is similar to that of chylomicron and VLDL particles, with lipolysis via LPL as a first step, followed by tissue uptake of remnant particles. As a multifunctional protein, LPL also functions as a bridge to mediate emulsion particle uptake independent of its hydrolytic activity, particularly for larger-size TGRPs [24–26]. In this study, using four types of lipid emulsions to mimic physiological CM and VLDL, we found that blood clearance of each emulsion particles was slowed in LPL+/- mice, and the effects of LPL reduction were less with CM-sized n-3 TG emulsions, but greater with VLDL-sized n-3 TG emulsions compared to n-6 TG emulsions of particles with the same size. Regarding tissue targeting, the reduced LPL expression in LPL+/- mice had almost no effect on n-3 TG emulsions with CM size but had more effects on those with VLDL size with respect to tissue uptake (fat, heart, muscle and liver) compared to n-6 TG emulsions. In vitro, LPL miRNA transfection differentially affected the catabolism of n-3 TG emulsions with CM- and VLDL-sized particles with

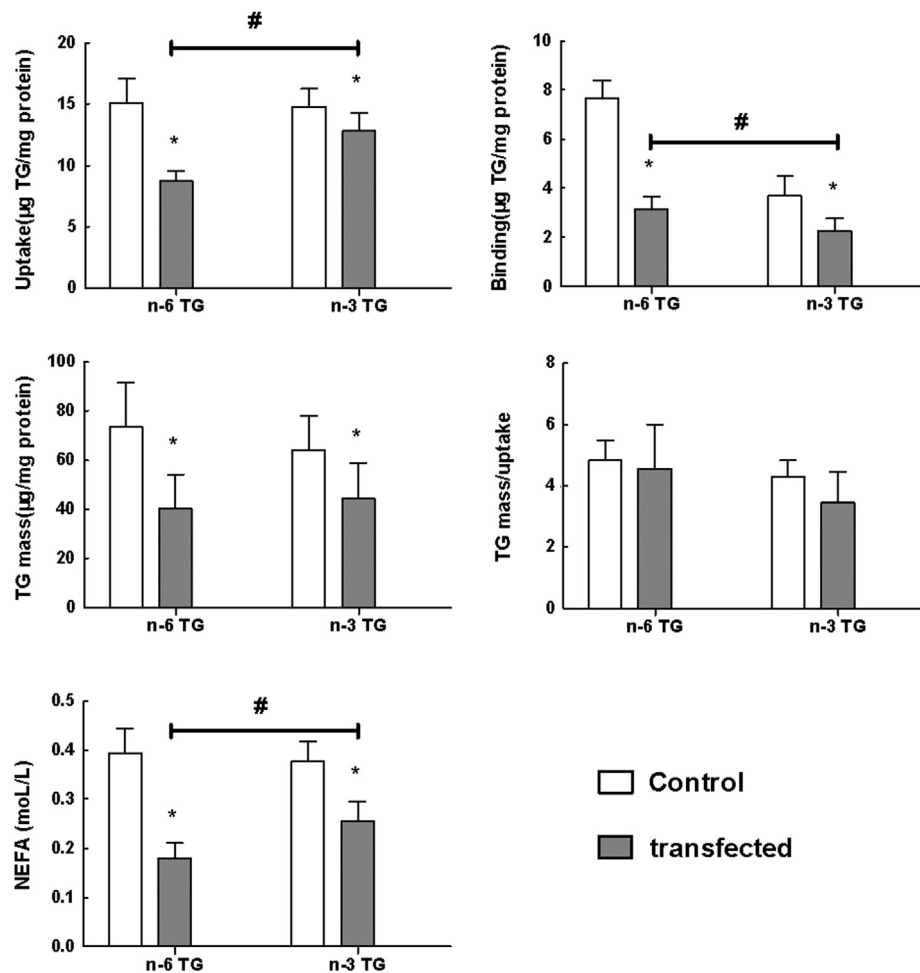


Fig. 3. Effects of lipoprotein lipase on the catabolism of CM-sized n-6 TG and n-3 TG emulsions in adipocytes: LPL miRNA-transfected 3T3-L1 cells were incubated with Dil-labeled emulsions with CM size (250 μg TG/mL) as indicated in Materials and Methods. After incubation, the cell TG mass, emulsion particle uptake and cell binding, and FFAs in medium were measured. The values represent the mean ± SD of triplicate measurements. *Indicates a significant difference from control ($P < 0.05$); #indicates a significant difference ($P < 0.05$) in changes between the n-6 and n-3 TG emulsions.

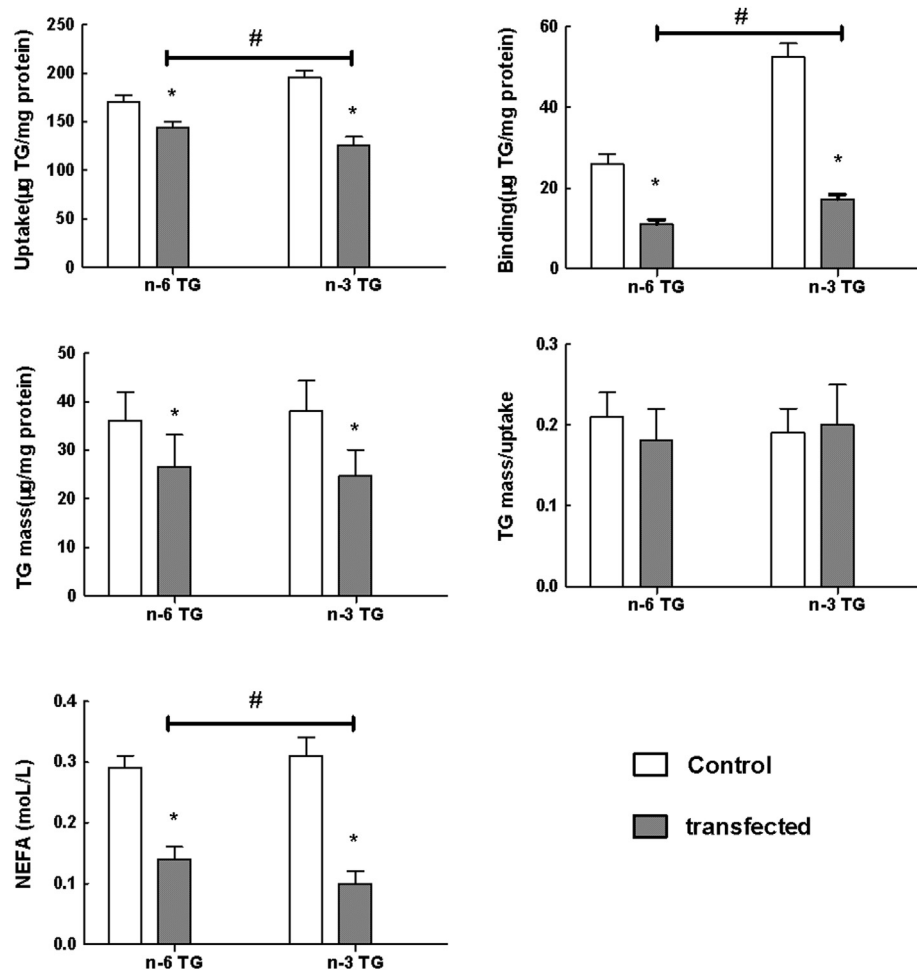


Fig. 4. Effects of lipoprotein lipase on the catabolism of VLDL-sized n-6 TG and n-3 TG emulsions in adipocytes: LPL miRNA-transfected 3T3-L1 cells were incubated with DiI-labeled emulsions with VLDL size (250 µg TG/mL) as indicated in Materials and Methods. After incubation, the cell TG mass, emulsion particle uptake and cell binding, and FFAs in medium were measured. The values represent the mean ± SD of triplicate measurements. *indicates a significant difference from control ($P < 0.05$); #indicates a significant difference ($P < 0.05$) in changes between n-6 and n-3 TG emulsions.

respect to particle uptake, cell TG mass, particle-cell binding and particle lipolysis. These results demonstrate lesser effects of the LPL on n-3 TG emulsions of CM-sized particles but greater effects on VLDL-sized particles compared to n-6 TG emulsions of particles of the same size.

The preference of LPL for different lipoproteins appears to be directly related to both the particle size and number; however, particle number may be more important in determining TGRP lipolysis and plasma clearance [27,28]. With a similar TG mass, larger particles were lipolyzed significantly less than smaller particles [28] and were removed faster from blood and taken up by tissues with less preceding lipolysis, particularly in the fed state [13], although differences in plasma clearance between the large- and small-size particles were reduced with equal numbers of particles administered [28]. Consistent with these findings, in the current study, we compared emulsions with different particle sizes, independent of n-3 TG or n-6 TG, and revealed that blood clearance of CM-sized emulsions was faster compared to emulsions of VLDL-sized particles. In terms of tissue targeting, the liver took up more CM-sized emulsion particles, whereas LPL-rich tissues and cells took up more VLDL-sized emulsion particles. In addition, the LPL expression in LPL+/- mice was reduced, and LPL miRNA-transfected adipocytes demonstrated greater effects on VLDL-sized emulsions with either n-3 TG or n-6 TG in blood clearance

and tissue uptake. Nevertheless, in clinical settings, lipid emulsions are always given to patients according to TG mass rather particle number and the design of intravenous lipid emulsions is based on large-size TGRP, chylomicrons, to reduce the length of their stay in blood circulation and increase TG utilization.

The effects of TG composition on the catabolism of TGRP have been studied in vitro and in vivo [29,30]. For example, inclusion of medium chain TG (MCT) into intravenous n-6 TG emulsions increases total lipolysis [29], but with markedly decreased hydrolysis of the n-6 TG component emulsions. This results from a displacement of n-6 TG from the particle surface by MCT, leading to greater solubility and mobility at the superficial water interface [30]. In vitro, hydrolysis of intravenous FO n-3 TG emulsions by both LPL and hepatic lipase is much less efficient than that of n-6 TG emulsions, and the release of EPA and DHA from n-3 TG is particularly inefficient [31]. In vivo, n-3 TG emulsions targeted less to LPL-rich tissues (i.e., adipose tissue and heart), and pre-injection of heparin or Triton WR 1339 had little or no effect on tissue uptake of n-3 TG emulsions but significantly reduced adipose tissue and heart uptake of n-6 TG emulsions with increased and reduced hepatic uptake, respectively [14,15]. Similar results were found in the current study using LPL+/- mice and LPL miRNA-transfected cells, indicating that a reduction in LPL expression decreased CM-sized n-3 TG emulsion uptake by adipose tissue and heart in vivo and

adipocytes in vitro to a lesser degree relative to n-6 TG emulsions. Thus, several other non-LPL mediated pathways may be involved in tissue and cellular uptake of n-3 TGRP, such as cellular surface proteoglycans, CD36, and phagocytosis [32,33].

Importantly, the findings that catabolism of n-3 TGRPs is less dependent on LPL were confined to CM-sized particles. However, for VLDL-sized TGRPs, we demonstrated that a reduction in LPL expression decreased n-3 TG emulsion uptake by adipose tissue and heart in vivo and to a greater extent by adipocytes in vitro, compared to n-6 TG emulsions [13]. Thus, unlike CM-sized particle emulsions, catabolism of n-3 TGRPs of VLDL size was more dependent on LPL.

The underlying mechanisms for the different effects of LPL on n-3 TG and n-6 TG emulsions with CM- and VLDL-sized particles are unknown. Whether modulators of LPL activity, such as activators (e.g., apoCII, apoE, apoAV), stabilizers (e.g., Gpihbp1) and inhibitors (e.g., apoCI, apoCIII, Angptl4) as well as modulators of LPL expression (e.g., VLDL receptor) [34,35] have different effects on LPL binding to TGRP with different TG and particle size needs to be elucidated.

5. Conclusions

Our data demonstrate that LPL acts differently on n-3 TGRP compared to n-6 TGRP depending on particle size. LPL plays more important roles in the catabolism of larger CM-sized n-6 TGRPs and VLDL-sized n-3 TGRPs, and the effects of LPL lessen with smaller-sized TGRPs. Catabolism of larger CM-sized n-3 TGRP may primarily rely on other pathways, such as cellular surface proteoglycans, CD36, and phagocytosis. Taken together, these findings may contribute to a better understanding of LPL and LPL-mediated lipolysis and the clearance of fish oil n-3 TG. These results may also be helpful for the development of novel therapies for hypertriglyceridemia and associated health problems.

Authors' contributions

LX carried out both animal and cell studies, and drafted the manuscript. CF carried out the cell miRNA transfection study and participated in the study design. HD participated in animal study and the statistical analysis. CW participated in both the cell and animal studies. YL participated in the animal study. RJD participated in the conceptual approaches, data analyses, and in reviewing the manuscript. KQ conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.clnu.2014.07.006>.

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