Effect of Alipogene Tiparvovec (AAV1-LPLS447X) on Postprandial Chylomicron Metabolism in Lipoprotein Lipase-Deficient Patients

André C. Carpentier, Frédérique Frisch, Sébastien M. Labbé, René Gagnon, Janneke de Wal, Stephen Greentree, Harald Petry, Jaap Twisk, Diane Brisson, and Daniel Gaudet

Department of Medicine, Division of Endocrinology (A.C.C., F.F., S.M.L.), Department of Pediatrics, Division of Genetics (R.G.), Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4; Amsterdam Molecular Therapeutics B.V. (J.d.W., S.G., H.P., J.T.), 1105 BA Amsterdam, The Netherlands; and ECOGENE-21 Clinical Research Center (D.B., D.G.), Department of Medicine, Université de Montréal, Chicoutimi, Canada H3G 1M8

Background: Lipoprotein lipase-deficient (LPLD) individuals display marked chylomicronemia and hypertriglyceridemia associated with increased pancreatitis risk. The aim of this study was to determine the effect of im administration of an adeno-associated viral vector (AAV1) for expression of LPLS447X in muscle (alipogene tiparvovec, AAV1-LPLS447X) on postprandial chylomicron metabolism and on nonesterified fatty acid (NEFA) and glycerol metabolism in LPLD individuals.

Methodology: In an open-label clinical trial (CT-AMT-011-02), LPLD subjects were administered alipogene tiparvovec at a dose of 1 × 10¹¹ genome copies per kilogram. Two weeks before and 14 wk after administration, chylomicron metabolism and plasma palmitate and glycerol appearance rates were determined after ingestion of a low-fat meal containing ³H-palmitate, combined with (continuous) iv infusion of [U-¹³C]palmitate and [1,1,2,3,3-²H]glycerol.

Principal Findings: After administration of alipogene tiparvovec, the triglyceride (TG) content of the chylomicron fraction and the chylomicron-TG/total plasma TG ratio were reduced throughout the postprandial period. The postprandial peak chylomicron ³H level and chylomicron ³H area under the curve were greatly reduced (by 79 and 93%, 6 and 24 h after the test meal, respectively). There were no significant changes in plasma NEFA and glycerol appearance rates. Plasma glucose, insulin, and C-peptide also did not change.

Conclusions/Significance: Intramuscular administration of alipogene tiparvovec resulted in a significant improvement of postprandial chylomicron metabolism in LPLD patients, without inducing large postprandial NEFA spillover. (J Clin Endocrinol Metab 97: 1635–1644, 2012)

Lipoprotein lipase deficiency (LPLD) is an autosomal recessive inherited disorder of lipid metabolism. This condition is characterized by reduced chylomicron triglyceride (TG) lipolysis and impaired chylomicron clearance from the circulation, leading to chylomicronemia and severe hypertriglyceridemia (1). Recurrent pancreatitis is the most debilitating and potentially lethal complication; other sequelae include increased tendency for atherosclerosis and diabetes (2). Diabetes may be caused by recurrent acute pancreatitis resulting in endocrine failure, but may also be linked to widespread lipoprotein lipase (LPL) malfunction resulting in broad metabolic disruption of energy uptake and distribution and overexposure of β-cells to fatty acids (3). Currently, clinical management of LPLD is based on severe dietary fat restriction and dietary supplementation with medium-chain fatty acids to minimize
TABLE 1. Characteristics of LPLD subjects at baseline and fasting levels of metabolites, hormones, and lipids before and after alipogene tiparvovec administration

<table>
<thead>
<tr>
<th>Subject no.</th>
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<th>TG (mmol/liter)</th>
<th>Glycerol (mmol/liter)</th>
<th>NEFA (µmol/liter)</th>
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P values are from unpaired Student’s t tests. M, Male; F, female.

* Diabetic subjects.

The aim of the present study was to investigate the effect of alipogene tiparvovec administration on postprandial chylomicron metabolism and plasma NEFA and glycerol appearance rates in LPLD patients. The postprandial study protocol was part of the clinical protocol for study CT-AMT-011-02 (ClinicalTrial.gov identifier: NCT00891306).

Subjects and Methods

Ethics statement

Informed written consent was obtained from all participants in accordance with the Declaration of Helsinki, and the protocol received approval from the Human Ethics Committee of the Institutional Review Board Services.

Subjects

The LPLD subjects were diagnosed and selected based on a history of pancreatitis, fasting plasma TG greater than 10 mmol/liter, a post-heparin LPL activity 20% or less of normal, and confirmed homozygosity or compound heterozygosity for mutations in the LPL gene. Regarding the latter, all five LPLD participants presented with a P207L mutation. None of the participants used oral lipid-lowering agents. Two subjects had insulin-dependent diabetes mellitus (participants 1001 and 1002). Results from these five LPLD subjects were compared with those of five overweight but otherwise healthy control subjects (Tables 1 and 2) who underwent assessment of postprandial chylomicron formation and to reduce chylomicron levels in the circulation.

A number of studies have been carried out to assess the safety and efficacy of a novel gene therapy approach (alipogene tiparvovec) for the treatment of adult LPLD patients (2). Alipogene tiparvovec contains the coding sequence for LPLS447X, a naturally occurring gain of function variant of LPL (4, 5) within a recombinant adeno-associated virus of serotype 1 (AAV1). Preclinical studies have shown that biologically active LPL can be produced by AAV1-mediated gene transfer to muscle (6, 7). Multiyear gene expression after a single (one-time) administration of adeno-associated vector (AAV) has also been shown, both in animal models (8, 9) and in humans (10, 11).

LPL is normally expressed mostly in adipose tissue, skeletal muscle, and myocardium (12, 13). In addition to stimulating chylomicron TG clearance toward adipose tissue fat storage, LPL activity in adipose tissue may significantly contribute to plasma nonesterified fatty acid (NEFA) appearance and exposure of nonadipose tissues to fatty acids (14). This NEFA spillover is exaggerated in prediabetic and diabetic subjects (15, 16) and appears to be mediated by adipose tissue LPL activity but not by skeletal muscle LPL activity (17). Alipogene tiparvovec is administered into adipose tissue LPL activity and confirmed homozygosity or compound heterozygosity for mutations in the LPL gene. Regarding the latter, all five LPLD participants presented with a P207L mutation. None of the participants used oral lipid-lowering agents. Two subjects had insulin-dependent diabetes mellitus (participants 1001 and 1002). Results from these five LPLD subjects were compared with those of five overweight but otherwise healthy control subjects (Tables 1 and 2) who underwent assessment of postprandial chylomicron formation and to reduce chylomicron levels in the circulation.

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The aim of the present study was to investigate the effect of alipogene tiparvovec administration on postprandial chylomicron metabolism and plasma NEFA and glycerol appearance rates in LPLD patients. The postprandial study protocol was part of the clinical protocol for study CT-AMT-011-02 (ClinicalTrial.gov identifier: NCT00891306).

TABLE 2. Characteristics of healthy controls at and fasting levels of metabolites, hormones, and lipids

<table>
<thead>
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<th>Subject no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>BMI (kg/m²)</th>
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M, Male; F, female; Nd, not determined; BMI, body mass index.
cron metabolism using similar methods except for meal fat content and tracer (see Postprandial study protocol).

Alipogene tiparvovec administration protocol (CT-AMT-011-02)

The five LPLD subjects were admitted to the ECOGENE 21 Clinical Research Center to receive alipogene tiparvovec (1 × 10^{12} gene copies per kilogram body weight) in a one-time series of multiple injections in skeletal muscles of both lower limbs under spinal analgesia. Oral immunosuppressive therapy with cyclosporine (3 mg/kg under spinal analgesia) and mycophenolate mofetil (2 g/d) was initiated 3 d before alipogene tiparvovec administration and continued for 12 wk to reduce possible immune reactivity against AAV1 capsids. A single iv injection of methylprednisolone (1 mg/kg) was administered 30 min before alipogene tiparvovec administration.

During at least 4 wk of “run-in” before, and up to the 14 wk after alipogene tiparvovec administration, subjects were monitored for signs and symptoms of LPLD and for safety and efficacy of the treatment. Subjects remained on a low-fat diet (<15% of daily calories in the form of fat) throughout the entire study period.

Postprandial study protocol

The participants of CT-AMT-011-02 underwent a metabolic study to determine postprandial chylomicron metabolism up to 24 h and plasma NEFA and glycerol appearance rate over 9 h after ingestion of a standard liquid test meal for 2 wk before (during the run-in) and 14 wk after alipogene tiparvovec administration. These metabolic studies were performed at the ECOGENE 21 Clinical Research Center in Chicoutimi according to standard operating procedures developed by the Human Metabolic Investigation Unit at the Centre de Recherche Clinique Etienne-Le Bel in Sherbrooke. Insulin and antihypertensive medications were withheld from relevant subjects in the morning of the metabolic study and resumed 9 h after ingestion of the test meal. All subjects received precooked low-fat meals for the 2 d preceding the metabolic study to ensure similar fat intake per patient and per time point.

On arrival, after a 12-h overnight fast, body weight and height were measured to calculate body mass index. An iv catheter was placed in one forearm for infusions, and another was placed in the contralateral arm maintained in a heating pad (≈55 °C) for blood sampling.

The standard liquid meal was prepared using a modification of our previously published protocol (16) to ensure a fat content appropriate for LPLD subjects (thereby limiting the risk of inducing a pancreatitis event). In brief, soybean oil (15 g/liter), safflower oil (9 g/liter), dried nonfat milk (280 g/liter), egg phospholipids (0.18 g/liter), and water with the addition of chocolate syrup (190 ml/liter) to provide 896 kcal with 13 g of fat (13% of energy) and 141 g of carbohydrates (65% of energy) were combined in a liquid meal of 500 ml, which was ingested over a 30-min period. One subject (no, 1003) was lactose intolerant, and therefore dried nonfat milk was replaced with soy milk (total volume of 700 ml instead of 500 ml). The fatty acid composition was similar to the composition of Intralipid, an iv fat emulsion used in previous studies: palmitate 10%, oleate 32%, linoleate 46%, α-linolenate 8%, and stearate 4% (19). 3H-Palmitic acid (300 µCi; Perkin-Elmer Inc., Woodbridge, Ontario, Canada) was sonicated into the liquid meal. Control subjects participated in a separate postprandial study in which a standard liquid meal containing 906 kcal with 31 g of fat (33% of energy, same fatty acid composition as above), 50% of energy as carbohydrates, and 3H-triolein (300 µCi; Perkin-Elmer Inc.) was ingested over 30 min. Oleate is slightly more rapidly absorbed and incorporated into chylomicrons than palmitate (20), potentially leading to a slight overestimation of the chylomicron peak and early postprandial area under the curve (AUC) chylomicron tracer activity in the controls vs. the LPLD subjects. All liquid meals were sampled to determine the dose of 3H-tracer administered per subject.

From 1 h before to 9 h after the ingestion of the meal, a constant infusion of [U-13C]K palmitate (0.01 µmol/kg·min in 100 ml 25% human serum albumin; Cambridge Isotopes Laboratories Inc., Andover, MA) and a primed (1.6 µmol/kg) continuous (0.05 µmol/kg·min) infusion of [1,1,2,3,3-2H]glycerol (Cambridge Isotopes Laboratories Inc.) were administered as described previously (16). The choice of a palmitate tracer in our experimental protocols was based on the following: 1) palmitate, oleate, and linoleate are the most prevalent NEFA in human plasma and have similar clearance rates in humans (21); 2) a palmitate tracer was previously used to measure total NEFA turnover in humans after oral fat intake (17, 22); and 3) use of palmitate tracer alone allows prediction of NEFA appearance as determined using combined palmitate and linoleate tracers during iv infusion of heparin + Intralipid in humans (r = 0.90; P < 0.001) (23). All tracers were pretested for sterility and nonpyrogenicity. Aliquots of the tracer solutions were sampled to determine the quantity of tracers administered.

Blood samples were taken at 1-h intervals, from 1 h before to 9 h after ingestion of the meal. A light meal without fat was given to the participants at the 9-h time point, and the final blood sample was taken after 24 h. Blood samples were taken every hour over 6 h during the postprandial state in control subjects. Blood was collected in tubes containing Na2EDTA and orlistat (30 µg/ml; Roche, Mississauga, Canada) to prevent in vitro TG lipolysis, and separate samples were collected in NaF tubes for plasma glucose determination (24).

Laboratory assays

All laboratory assays were performed at the Human Metabolic Investigation Unit in Sherbrooke. Glucose, insulin, total NEFA, TG, and chylomicron TG were measured as described (15). Plasma glycerol and plasma and infused [1,2,3,3-2H]glycerol content were measured by gas chromatography-mass spectrometry, whereas plasma palmitate, linoleate, oleate, and plasma and infused [U-13C]palmitate concentrations were measured by liquid chromatography-mass spectrometry after lipid extraction as previously described (24). Chylomicrons were separated by two sequential ultracentrifugations for 15 min at 33,000 rpm at 20 °C (Beckman Optima L-100XP using a 100Ti rotor; Beckman Coulter, Fullerton, CA). Total plasma, chylomicron, and lipid meal 3H activity was determined by scintillation counting (Beckman Coulter LS6500) after lipid extraction using ethanol and ether (3:1), evaporation, and resuspension in diethyl ether. Total plasma 3H activity represents the sum of tracer activity in chylomicrons and its redistribution in NEFA, very low-density lipoprotein, and its daughter lipoproteins (remnants). It also includes some 3H activity in water after oxidation of the tracer. The intra- and interassay coefficients of variation were less than 15% for all assays.
Calculations

Plasma palmitate and glycerol appearance rates were calculated using Steele’s non-steady-state equation, assuming a volume of distribution of 90 ml/kg and 230 ml/kg, respectively (25–27). Plasma and chylomicron $^3$H activities were expressed as percentage of ingested dose (%ID)/100 ml of plasma, and postprandial AUC was calculated using the trapezoidal method. In subject 1002, venous access was interrupted between 7 and 9 h during the metabolic study carried out 2 wk before alipogene tiparvovec administration. In subject 2002, samples taken 14 wk after alipogene tiparvovec were largely destroyed during transport. Chylomicron-$^3$H activity was therefore measured for the missing time points for this subject by isolating chylomicrons from retained (frozen) samples. All subjects had a blood sample taken 24 h after ingestion of the test meal to calculate chylomicron $^3$H activity at 24 h. Because postprandial data in our control group were collected over 6 h, we also calculated postprandial plasma and chylomicron $^3$H activity AUC over 6 h for comparison with this group.

Statistical analyses

Data are expressed as mean ± SD in the text and in the tables and as mean ± SEM in the figures, unless stated otherwise. Intragroup characteristics were compared by paired Student’s t test or two-way ANOVA for repeated measures in the case of postprandial curves with pretreatment vs. posttreatment, postprandial time, and interaction as independent variables. A two-tailed $P$ value < 0.05 was considered significant. All analyses were performed with SAS software for Windows, version 9.1.3 (SAS Institute Inc., Cary, NC).

Results

Effect of alipogene tiparvovec on fasting plasma metabolites, hormones, and lipids (Tables 1 and 2)

All LPLD subjects displayed chylomicronemia and very high fasting plasma TG levels [mean ± sd, 18.8 ± 8.7 mmol/liter in LPLD subjects (n = 5) vs. 1.1 ± 0.4 mmol/liter in controls (n = 5); Tables 1 and 2]. Two subjects (no. 1001 and 1002) had mild to moderate fasting hyperglycemia.

Plasma glucose ($P = 0.05$) and insulin ($P = 0.19$) tended to be increased 14 wk after alipogene tiparvovec administration compared with 2 wk before administration, whereas fasting plasma NEFA, C-peptide, and glycerol levels did not change (Tables 1 and 2). Fasting plasma TG levels tended to be reduced in four of the five subjects ($P = 0.12$) 14 wk after administration. As for plasma NEFA, fasting plasma palmitate levels remained unchanged after vs. before administration ($207 ± 105$ vs. $203 ± 56 \mu$mol/liter, respectively; $P = 0.92$).

Postprandial glucose, insulin, and C-peptide (Fig. 1) and total plasma and chylomicron TG levels (Fig. 2)

Plasma glucose excursion (Fig. 1A) was similarly abnormal in LPLD subjects (mean peak glucose level over 10 mmol/liter at 1 h) both before and 14 wk after alipogene tiparvovec administration (ANOVA $P = 0.34$ for effect of treatment) compared with control subjects. This increase in glucose level was driven by two diabetic subjects. Alipogene tiparvovec did not significantly affect postprandial insulin (Fig. 1B) and C-peptide (Fig. 1C) excursions (ANOVA $P = 0.33$ and $P = 0.50$, respectively).

Mean total plasma TG levels in LPLD subjects, which were persistently high throughout the postprandial period before administration (Fig. 2A), were significantly reduced (by ~60%) 14 wk after alipogene tiparvovec ad-
administration (ANOVA \( P < 0.001 \)). Mean chylomicron TG levels over the duration of the postprandial period (Fig. 2B), representing the majority of TG in plasma, were also significantly reduced (by \(-85\%) after alipogene tiparvovec administration (ANOVA \( P < 0.001 \)). Average buoyancy of plasma TG, as assessed by the chylomicron TG/total plasma TG ratio (Fig. 2C), was reduced in every LPLD subject (ANOVA \( P < 0.001 \)) throughout the postprandial period 14 wk after alipogene tiparvovec administration [from 0.64 (range, 0.55 to 0.84) before administration to 0.15 (range, 0.00 to 0.43) after alipogene tiparvovec administration].

Postprandial plasma and chylomicron \(^3\)H activity curves and AUC (Figs. 3 and 4)

The postprandial plasma \(^3\)H activity curve 2 wk before administration was grossly elevated in every LPLD subject and did not reach a peak or plateau until 9 h after ingestion of the radio-labeled test meal (Fig. 3A). In marked contrast, 14 wk after alipogene tiparvovec administration, the postprandial plasma \(^3\)H activity curves were greatly reduced, with evidence of a plateau or peak well within 9 h in every LPLD subject (Fig. 3B). This resulted in an average postprandial plasma \(^3\)H activity curve that was significantly reduced after vs. before alipogene tiparvovec administration (ANOVA \( P < 0.001 \)) (Fig. 3C) and a significantly different postprandial time \( \times \) treatment interaction term (\( P = 0.03 \)). The first 6 h of the postprandial plasma \(^3\)H curve (Fig. 3C) and AUC (Fig. 3D) were almost superimposable on that of healthy control subjects. The 24-h AUC for postprandial plasma \(^3\)H activity was reduced by more than 2-fold (before vs. after administration, 1119 \( \pm \) 366 and 534 \( \pm \) 64\%ID/100 ml plasma \( \times \) 1440 min at 14 wk, respectively; mean \( \pm \) sd; \( P = 0.07 \)).

The postprandial chylomicron \(^3\)H activity curve was also grossly abnormal in every LPLD subject before administration and did not reach a peak or plateau until 9 h after ingestion of the radio-labeled test meal. \(^3\)H activity in the chylomicron fraction was very high up to 24 h after ingestion (Fig. 4A). In contrast, 14 wk after alipogene tiparvovec administration, the postprandial chylomicron \(^3\)H activity curve was strikingly reduced, with evidence of peaking well within 9 h in every LPLD subject (Fig. 4B). This resulted in a significantly reduced average chylomicron \(^3\)H activity curve (ANOVA \( P < 0.001 \)) (Fig. 4C) and a significantly different postprandial time \( \times \) treatment interaction term (\( P < 0.001 \)) compared with before alipogene tiparvovec administration. The AUC for average chylomicron \(^3\)H activity over the first 6 h after the test meal was reduced by 79\% (range, 65 to 91\%); \( P = 0.03 \)) (Fig. 4D). The first 6 h of the postprandial chylomicron \(^3\)H activity curve and AUC were almost superimposable on that of the healthy controls (Fig. 4, C and D). The 24-h AUC for postprandial chylomicron \(^3\)H activity was significantly reduced by 93\% (range, 89 to 98\%) (before vs. after administration, 678 \( \pm \) 280 and 40 \( \pm \) 14\%ID/100 ml plasma \( \times \) 1440 min at 14 wk, respectively; mean \( \pm \) sd, \( P = 0.03 \)).
Postprandial plasma NEFA and glycerol levels and appearance rates (Fig. 5)

Postprandial plasma NEFA (Fig. 5A) and glycerol levels (Fig. 5C), which reached a nadir within 2 h and gradually returned to fasting values within 9 h, did not change significantly after alipogene tiparvovec administration (ANOVA \( P = 0.08 \) and \( P = 0.96 \), respectively). There was also no significant difference between pre- and postadministration postprandial plasma palmitate (Fig. 5B) and glycerol (Fig. 5D) appearance rates (ANOVA \( P = 0.24 \) and \( P = 0.65 \), respectively).

Discussion

The present study shows that a one-time administration of alipogene tiparvovec (which results in AAV1-mediated LPLS447X expression in skeletal muscle) promotes postprandial chylomicron metabolism in LPLD patients. NEFA and glycerol appearance rates are not affected. At baseline, all five LPLD participants showed severe hypertriglyceridemia (plasma TG > 10 mmol/liter), with most of the TG circulating as large/buoyant chylomicrons. After ingestion of the test meal, all five LPLD subjects had a profound delay in chylomicron clearance, as reported previously (28). Fourteen weeks after alipogene tiparvovec administration, plasma TG levels were reduced in four of the five LPLD subjects, with a more robust reduction of TG within the large/buoyant chylomicron fraction. As a result, the overall buoyancy of the TG-rich lipoprotein pool was significantly affected, as demonstrated by a reduced chylomicron-TG/total TG ratio throughout the postprandial period. After alipogene tiparvovec administration, all five subjects displayed improved postprandial metabolism of newly formed chylomicrons demonstrated by a reduced peak level and reduced AUC for postprandial chylomicron \(^3\)H activity after ingestion of the test meal. After administration, the LPLD subjects displayed a postprandial chylomicron metabolism similar to that of healthy controls.

In previous clinical studies (CT-AMT-010-01 and CT-AMT-011-01), administration of alipogene tiparvovec to LPLD subjects resulted in a reduction of median fasting total plasma TG in the majority of subjects (comparing data obtained in the first 12 wk after administration to preadministration data), with a return to baseline level beyond 12 wk (2, 4, 29). Despite transient effects on total plasma TG, LPL expression was demonstrated up to 26 wk in the injected muscles. In addition, there was a clinically significant decrease in the incidence of pancreatitis during the follow-up period (2, 4), indicating persistent...
transgene expression and clinical effect. The current results suggest that the predominant effect of alipogene tiparvovec is on the metabolism of newly formed, large, buoyant chylomicrons, with less effect on remnant chylomicrons and very low-density lipoprotein and, therefore, less effect on total plasma TG. We hypothesize that the improved metabolism of newly formed chylomicrons is instrumental in the observed reduction in pancreatitis.

Skeletal muscle was chosen as the injection site because: 1) it is an easily accessible tissue for administration; 2) we and others have shown that muscle, but not adipose tissue, is amenable to AAV-mediated gene transfer and transduction, as noted in both preclinical and clinical studies (7, 18); 3) skeletal muscle is a natural site of LPL production; 4) despite the presence of some intramyocellular TG accumulation, im administration of AAV1-LPLS447X has not led to gross local lipotoxicity in preclinical animal models or in LPLD patients as assessed by the absence of muscle pain and weakness and normal circulating creatine phosphate kinase levels (4, 6, 29). A biopsy taken from an injected muscle 1 yr after gene transfer tested positive for vector DNA sequence, LPL protein, and LPL activity (Ferreira V., J. Twisk, K. Kwikkers, E. Aronica, C. E. Hack, S. Greentree, D. Brisson, J. Methot, H. Petry, and D. Gaudet, unpublished observations). Preliminary results from postprandial studies carried out 1 yr after gene transfer (carried out in three of the five subjects included in the current study) also indicated continued biological effect up to this point. Based on available results, we can therefore conclude that the effect lasts up to 1 yr after gene transfer, at least in some LPLD subjects.

Despite major improvement in postprandial chylomicron metabolism, we did not observe an increase in postprandial plasma NEFA and glycerol levels or appearance rates 14 wk after administration of alipogene tiparvovec. Similarly, postprandial glucose, insulin, and C-peptide levels did not appear to be affected by alipogene tiparvovec. Postprandial plasma NEFA and glycerol appearance are thought to occur as a result of two distinct processes that take place mostly in adipose tissue: 1) intracellular adipose tissue TG lipolysis; and 2) plasma NEFA appearance from LPL-mediated extracellular lipolysis of TG-rich lipoproteins (the so-called “NEFA spillover”) (17, 30–34). NEFA and glycerol appearance rates during iv lipid infusion or during the postprandial state are mostly driven by excess adiposity in humans (16, 35). We have shown that excess NEFA appearance during iv lipid infusion in prediabetic subjects is a function of NEFA spillover (a LPL-mediated phenomenon) and not intracellular lipolysis (15). In the postprandial state, uptake of NEFA generated by chy-
Lomicron TG hydrolysis across skeletal muscle circulation is 100%, in marked contrast with net spillover of NEFA from chylomicron TG from the adipose tissue (17). Alipogene tiparvovec is injected im, and LPL expression and activity occurs predominantly in muscle after such administration (6). Our results are therefore compatible with very efficient tissue extraction of NEFA and glycerol from chylomicron-TG hydrolysis by LPL-expressing skeletal muscles, resulting in very low NEFA and glycerol spillover into the systemic circulation because adipose tissue LPL activity is still abolished. The lower postprandial plasma NEFA levels in LPLD subjects (vs. healthy subjects), before as well as after alipogene tiparvovec administration, further supports this interpretation. Future studies are needed to ascertain the postprandial tissue partitioning of dietary fatty acids after alipogene tiparvovec administration.

The present postprandial study is limited by its small sample size and open-label design. The small sample size of the present study is due to the rarity of LPLD subjects who suffered from a previous pancreatitis episode and thus were eligible for LPL gene replacement therapy. Although the lack of statistical significance should be interpreted with caution because of the likelihood of type II error due to the small sample size, we nevertheless found the primary outcome (change in chylomicron metabolism in response to treatment within the LPLD group) to be significantly changed. The very large treatment effect on primary outcomes (postprandial chylomicron TG levels and chylomicron $^{3}$H excursion) and prospective design with repeated measures that allow within-subject comparisons helps to compensate for the small sample size. Furthermore, we applied tracer techniques that directly addressed postprandial metabolism of (newly formed) chylomicron particles and NEFA and glycerol appearance rates during the postprandial state. One limitation of oral administration of labeled fatty acids is that this technique and the interpretation of the data assume total and efficient intestinal fat absorption, which may not be the case, especially in individuals with antecedent pancreatitis and possible impairment of exocrine pancreatic function. This limitation is unlikely to be relevant for the assessment of treatment effect in LPLD subjects (within-subject comparison) but may certainly limit the ability to compare (treated and untreated) LPLD vs. controls. Because of this potential limitation, we referred to “chylomicron metabolism” instead of “chylomicron clearance” in our conclusion and displayed tracer results for the control group without formal statistical comparison with the LPLD group to avoid over-interpretation of the present results. The risk of a type II

![FIG. 5. Postprandial plasma NEFA level (A), palmitate appearance rate (B), glycerol level (C), and glycerol appearance rate (D) are shown in LPLD subjects 2 wk before (closed circles) and 14 wk after (open circles) alipogene tiparvovec administration. Postprandial NEFA levels in healthy control subjects are also shown (controls, closed squares). Data are expressed as mean ± SEM. Ra, Appearance rate.](image-url)
error is high with regard to our conclusion on the (lack of) effect of alipogene tiparvovec on postprandial NEFA and glycerol appearance rates due to the very small sample size. However, comparison of the postprandial plasma NEFA response of LPLD subjects with that of the controls clearly demonstrated reduced NEFA levels in the postprandial phase in the former group before and after alipogene tiparvovec administration. This supports our conclusion that alipogene tiparvovec does not induce a significant rebound in postprandial NEFA spillover.

The response of chylomicron metabolism to alipogene tiparvovec in the two diabetic subjects (no. 1001 and 1002) (Fig. 4, A and B) was at least as positive as for the other three LPLD subjects. Therefore, we believe that inclusion of these two diabetic subjects did not affect inference with regard to the primary outcome of our study. Although not statistically significant, both fasting and postprandial glucose levels tended to be higher after alipogene tiparvovec treatment in LPLD subjects. There was also a nonsignificant trend toward increased fasting insulin level, although postprandial insulin excursion was very similar after vs. before treatment. Because of the small number of subjects, we cannot rule out that these trends represent true deterioration in glucose homeostasis. Furthermore, we cannot determine the mechanism for this effect because we did not measure plasma glucose production rate and did not formally assess the degree of insulin sensitivity in these subjects.

In conclusion, the present study demonstrates that alipogene tiparvovec markedly improves postprandial chylomicron metabolism without inducing a large increase in plasma NEFA and glycerol appearance rates. The overall result is a much reduced postprandial level of newly formed, large/buoyant chylomicrons, which are thought to be the most pathogenic and causal in eliciting acute (recurrent) pancreatitis in LPLD subjects.

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Address all correspondence and requests for reprints to: Dr. André Carpentier, Division of Endocrinology, Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4. E-mail: andre.carpentier@usherbrooke.ca. Or Dr. Daniel Gaudet, ECOGENE-21, Chicoutimi Hospital, Department of Medicine, Université de Montréal, Québec, Canada G7H 5H6. E-mail: daniel.gaudet@umontreal.ca.

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