The *Hyplip2* locus causes hypertriglyceridemia by decreased clearance of triglycerides[S]

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Abstract The Hyplip2 congenic mouse strain contains part of chromosome 15 from MRL/MpJ on the BALB/cJ background. Hyplip2 mice show increased plasma levels of cholesterol and predominantly triglycerides (TGs) and are susceptible to diet-induced atherosclerosis. This study aimed at elucidation of the mechanism(s) explaining the hypertriglyceridemia. Hypertriglyceridemia can result from increased intestinal or hepatic TG production and/or by decreased LPL-mediated TG clearance. The intestinal TG absorption and chylomicron formation were studied after intravenous injection of Triton WR1339 and an intragastric load of olive oil containing glycerol tri[^{3}H]oleate. No difference was found in intestinal TG absorption. Moreover, the hepatic VLDL-TG production rate and VLDL particle production, after injection of Triton WR1339, were also not affected. To investigate the LPL-mediated TG clearance, mice were injected intravenously with glycerol tri[^{3}H]oleate-labeled VLDL-like emulsion particles. In Hyplip2 mice, the particles were cleared at a decreased rate (half-life of 25 ± 6 vs. 11 ± 2 min; $P < 0.05$) concomitant with a decreased uptake of emulsion TG-derived ^{3}H-labeled fatty acids by the liver and white adipose tissue. The increased plasma TG levels in Hyplip2 mice do not result from an enhanced intestinal absorption or increased hepatic VLDL production but are caused by decreased LPL-mediated TG clearance.— Moen, C. J. A., A. P. Tholens, P. J. Voshol, W. de Haan, L. M. Havekes, P. Gargalovic, A. J. Lusis, K. Willems van Dyk, R. R. Frants, M. H. Hofker, and P. C. N. Rensen. **The *Hyplip2* locus causes hypertriglyceridemia by decreased clearance of triglycerides. *J. Lipid Res.* 2007.** 48: **2182–2192.**

Supplementary key words congenic mice • lipoproteins • lipoprotein lipase • triglyceride hydrolysis • lipoprotein clearance

The metabolic syndrome is a collection of risk factors that increases the risk of developing type 2 diabetes, heart disease, or stroke. One of these risk factors is combined hyperlipidemia. Genetic as well as environmental factors contribute to this disease. A powerful approach to identify genes underlying complex phenotypes like hyperlipidemia is by using hyperlipidemic animal models and then testing the relevance of those identified genes in human populations.

Using linkage analysis on several mouse genetic crosses, an interesting quantitative trait locus for lipoprotein metabolism has been found on mouse chromosome 15 (1–5). This *Hyplip2* locus influences cholesterol and triglyceride (TG) levels in plasma. The most striking linkage at this locus was found in a cross between the inbred strains MRL/MpJ (MRL) and BALB/cJ (B/c) (2). To characterize and eventually isolate the underlying *Hyplip2* gene, a speed-congenic strain has been produced using marker-assisted backcrossing (6). This Hyplip2 congenic strain carries a part of chromosome 15 from the inbred strain MRL on the homogeneous genetic background of the inbred strain B/c. Analysis of these congenic mice showed increased total cholesterol and TG levels on chow and high-fat diets after overnight fasting (6). The Hyplip2 congenic mouse, therefore, represents a valuable model to facilitate the functional and biochemical characterization of the underlying gene(s).

The aim of this study was to evaluate in detail the role of the *Hyplip2* locus in TG metabolism. Therefore, we studied

Manuscript received 8 January 2007 and in revised form 29 May 2007 and in re-revised form 28 June 2007.

Published, JLR Papers in Press, July 3, 2007.
DOI 10.1194/jlr.M700009-JLR200

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one Table.

the effect of *Hyplip2* on intestinal TG absorption, VLDL-TG production and clearance, and tissue-specific uptake of FFAs derived from VLDL-TG and from the plasma FFA pool. We show that the *Hyplip2* locus causes hypertriglyceridemia by decreased clearance of TG from the plasma and found a decreased uptake of VLDL-TG-derived FFAs in adipose tissue but not in other peripheral tissues. The *Hyplip2* locus was found to act by modulation of the LPL activity specifically, because clearance of albumin-bound FFAs from the circulation was not affected by Hyplip2.

From this study, we conclude that the hypertriglyceridemia observed in the Hyplip2 mouse is caused by decreased LPL-mediated hydrolysis of TG-rich lipoproteins.

EXPERIMENTAL PROCEDURES

Animals

Congenic Hyplip2 mice were generated as described previously (6). B/c mice were purchased from Jackson Laboratories (Bar Harbor, ME). For all experiments, female mice were used. The mice were housed under standard conditions with a 12 h light cycle (7:00 AM–7:00 PM) and were fed a regular mouse diet [RM3 (E) DU; Special Diet Services, Witham, England] and given free access to food and water. Experiments were performed in the fed state at 8:00 AM and/or after 4 h of fasting at 12:00 AM with food withdrawal at 8:00 AM. All animal experimental protocols were approved by the Committee on Animal Experimentation of the Leiden University Medical Center.

Measurement of food intake

Food intake was assessed by automated measurements in metabolic cages (Oxymax Comprehensive Lab Animal Monitoring System; Columbus Instruments, Columbus, OH). One animal was kept in each cage. Registrations were started after the mice had been adapted to the cage for 1 day. In the metabolic cages, the mice had free access to powdered regular mouse diet and tap water. The accumulated food intake was measured over the complete light and dark phases for 2 days.

Plasma lipid and lipoprotein analysis

Blood was collected by tail bleeding into chilled paraoxon (Sigma, St. Louis, MO)-coated capillary tubes to prevent ongoing in vitro lipolysis (7), unless indicated otherwise. The tubes were placed on ice and centrifuged, and the plasma was assayed for total cholesterol, TG, and FFA using commercially available enzymatic kits (Nos. 11489437216 and 11488872216; Roche Diagnostics, Almere, The Netherlands, and NEFA-C kit 999-75406; Wako Chemicals GmbH, Neuss, Germany, respectively). For determination of the lipid distribution over plasma lipoproteins by fast-protein liquid chromatography, 50 μl of pooled plasma from eight mice per group was injected onto a Superose 6 HR 10/30 column (Äkta System; Amersham Pharmacia Biotech, Piscataway, NJ) and eluted at a constant flow rate of 50 μl/min PBS and 1 mM EDTA (Sigma), pH 7.4. Fractions of 50 μl were collected and assayed for total cholesterol and TG as described above.

Hepatic VLDL-TG production

Mice were anesthetized by intraperitoneal injection of acepromazine (6.25 mg/kg; Neurotranq Alvasan International BV, Weesp, The Netherlands), dormicum (6.25 mg/kg; Roche Netherlands, Mijdrecht, The Netherlands), and fentanyl (0.31 mg/kg; Janssen-Cilag BV, Tilburg, The Netherlands) and injected via the tail vein with trans-^{35}S label (150 μCi/mouse). After 30 min, 500 mg of Triton WR1339 (Sigma) per kilogram of body weight was injected to block lipolysis (8). Blood samples were drawn at 10, 30, 60, and 90 min after administration via tail bleeding. At 120 min, mice were exsanguinated and the VLDL fraction of each mouse was isolated quantitatively from 200 μl of plasma after density gradient ultracentrifugation (9). VLDL-TG was measured as described above. VLDL-apolipoprotein B (apoB) was precipitated selectively by 2-propanol (10) and counted for the incorporated ^{35}S.

Intestinal TG absorption

To measure intestinal lipid absorption, overnight-fasted mice received an intravenous injection of Triton WR1339 (0.5 mg/g, 10% solution in PBS; Sigma) to block lipoprotein clearance (11). Subsequently, mice received an intragastric load of glycerol tri[^{3}H]oleate ([^{3}H]TO) (12 μCi; Amersham Biosciences) and [^{14}C]oleate (3.3 μCi; Amersham Biosciences) in 200 μl of olive oil (Carbonell). Blood samples were drawn before the gavage (time 0) and at 0.5, 1, 2, 3, and 4 h after the gavage. Plasma TG was measured as described above, and plasma ^{3}H and ^{14}C activities were counted in 2 ml of Ultima Gold (Packard Bioscience, Meriden, CT). We confirmed that >90% of the radioactivity measured in plasma was present in the TG fraction.

Postprandial TG response

To determine the effect of *Hylip2* on the postprandial TG response, 4 h-fasted mice received an intragastric load of 200 μl of olive oil (Carbonell). Blood samples of 35 μl were drawn as described above just before the gavage (time 0) and at 1, 2, 4, and 6 h after the gavage. Obtained plasma samples were assayed for TG as described above.

Preparation of VLDL-like TG-rich emulsion particles

The preparation and characterization of 45 nm protein-free VLDL-like emulsion particles have been described (12). Briefly, emulsion particles were prepared by sonication from 100 mg of total lipid at an egg yolk phosphatidylcholine (Lipoid, Ludwigshafen, Germany)-glycerol trioleate-lysophosphatidylcholine-cholesteryl oleate-cholesterol (all from Sigma) weight ratio of 22.7:70:2.3:3.0:2.0 in the presence of 50 μCi of [^{3}H]TO and 10 μCi of cholesteryl [^{14}C]oleate ([^{14}C]CO; Amersham Biosciences) using a Soniprep 150 (MSE Scientific Instruments, Crawley, UK) at 10 μm output. Emulsions were stored at 4°C under argon and used within 3 days.

In vivo lipolysis

Bolus experiment (acute). To study the acute in vivo plasma clearance of the radiolabeled emulsion particles, mice were anesthetized as described above and the abdomens were opened. The emulsion (1 mg of TG) was injected intravenously via the vena cava inferior. Blood samples (<50 μl) were taken via the vena cava inferior at the indicated times, and the radioactivity in plasma was counted in 2.5 ml of Ultima Gold. The total plasma volumes of the mice were calculated from the equation V (ml) = 0.04706 × body weight (g), as determined from ^{125}I-BSA clearance studies as described previously (13). After 15 min, mice were euthanized and organs were isolated and dissolved in Soluene (70°C, overnight). Radioactivity was counted in 15 ml of Ultima Gold.

Infusion experiment (steady state). Mice were anesthetized as described above, and an infusion needle was inserted into the

tail vein and connected to a Harvard microdialysis low-flow 11 minipump (Holliston, MA). Infusion of [^{3}H]TG-labeled emulsion particles (1.0 mg TG/ml) and a trace amount of [^{14}C]palmitic acid (Amersham) complexed to BSA (2%) was started in the presence of citrate (3 μg/ml) at a rate of 0.2 ml/h for 2 h to achieve steady-state TG levels. After 1.5 and 2 h, a 150 μl blood sample was taken by tail bleeding. Subsequently, the mice were euthanized and their organs were quickly removed and frozen in liquid nitrogen. Plasma levels of TG and FFA were determined as described above. Lipid extraction from plasma was performed according to Bligh and Dyer (14). The lipid fraction was dried under nitrogen, dissolved into chloroform-methanol (5:1, v/v), and subjected to TLC (LK5D gel 150; Whatman) using hexane-diethylether-acetic acid (83:16:1, v/v/v) as the mobile phase. Lipids were visualized by I_2 vapor and scraped off. Lipids were dissolved in hexane, and radioactivity was measured. Organs were dissolved in 5 M KOH in 50% (v/v) ethanol at 40°C for 3 days. Retention of TG-derived FAs and albumin-bound FAs in the tissues was calculated as nanomoles of FFA per milligram of protein, as determined from the specific activities of [^{3}H]FA and [^{14}C]FA in plasma.

Total lipase activity in plasma

To determine total plasma LPL activity levels, fasted mice were injected via the tail vein with heparin (0.1 U/g; Leo Pharmaceutical Products BV, Weesp, The Netherlands) and blood was collected after 10 min using heparin-coated capillaries. The plasma was snap-frozen and stored at −80°C until analysis of total LPL activity as modified from Zechner (15). In short, a TG substrate mixture containing TO (4.6 mg/ml), [^{3}H]TO (2.5 μCi/ml), essentially FA-free BSA (20 mg/ml; Sigma), Triton X-100 (0.1%; Sigma), and heat-inactivated (30 min at 56°C) human serum (20%) in 0.1 M Tris-HCl, pH 8.6, was generated by six sonication periods of 1 min using a Soniprep 150 at 7 μm output, with 1 min intervals in between on ice. Ten microliters of postheparin plasma was added to 0.2 ml of substrate mixture and incubated for 30 min at 37°C in the presence or absence of 1 M NaCl, which completely inhibits LPL activity, to estimate both LPL and HL levels. After incubation, 50 μl of the reaction mixture was added to 3.25 ml of heptane-methanol-chloroform (100:128:137, v/v/v) and 1 ml of 0.1 M K_2CO_3 in saturated H_3BO_3, pH 10.5, was added. To quantify the generated [^{3}H]oleate, 500 μl of the aqueous phase obtained after vigorous mixing and centrifugation (15 min, 3,600 rpm) was counted for ^{3}H activity in 4.5 ml of Ultima Gold. TG hydrolase activity was expressed as the amount of [^{3}H]oleate released per hour per milliliter of plasma. The fraction of TG hydrolase activity not inhibited by 1 M NaCl was considered the HL activity. LPL activity was calculated as the fraction of total TG hydrolase activity inhibited by 1 M NaCl.

Modulated lipolytic activity in plasma

To study the effect of *Hyplip2* on lipase activity in plasma in situ, postheparin plasma (1.4% of the incubation volume) was incubated with [^{3}H]TO-labeled VLDL-like protein-free emulsion particles (0.25 mg TG/ml) and excess FA-free BSA (60 mg/ml) in 0.1 M Tris, pH 8.5. After 1 h of incubation, 50 μl samples from the total 200 μl incubation volume were added to 1.5 ml of extraction liquid (methanol-chloroform-heptane-oleic acid, 1,404:1,245:1,001:1, v/v/v/v) and 0.5 ml of 0.2 M NaOH was added to terminate lipolysis. Generated [^{3}H]oleate was counted as described above, and the TG hydrolase activity was expressed as the amount of FA released per hour per milliliter. In this assay, the lipolytic activity of plasma is determined toward a relatively low amount of emulsion particles instead of an excess of solubilized TG. Therefore, the modulated lipolytic activity of plasma is assessed by allowing the interference of endogenous activators (e.g., apoC-II) and inhibitors (e.g., apoC-I, apoC-III, and angiopoietin-like proteins 3 and 4) with the activity of LPL.

Total lipase activity in tissues

To determine total lipase activity in tissues, tissue biopsies (100–150 mg) were taken from 4 h-fasted mice. The tissues were cut into small pieces and incubated for 1 h at 37°C in DMEM with BSA (2%) and heparin (2 U/ml) to release tissue-bound LPL. The samples were centrifuged for 10 min at 13,000 rpm, after which the supernatant was used for the assay of LPL activity, as modified from Zechner (15). In short, supernatant (10% of the incubation volume) was incubated with 200 μl of LPL substrate mixture containing 4.6 mg/ml triolein including glycerol-tri-(9,10-[^{3}H])oleate, heat-inactivated (1 h at 56°C) human serum (20%), Trition X-100 (0.1%), and FA-free BSA (20 mg/ml) in 0.1 M Tris, pH 8.6. After 30 min of incubation, 50 μl of the reaction mixture was added to 3.25 ml of heptane-methanol-chloroform (100:128:137, v/v/v) and 1 ml of 0.1 M K_2CO_3 in saturated H_3BO_3, pH 10.5, was added. Generated [^{3}H]oleate was counted as described above, and the TG hydrolase activity was expressed as the amount of FA released per hour per gram of tissue.

Effect of heat-inactivated mouse plasma on bovine LPL activity

To examine the effect of plasma components on bovine LPL activity, plasma was taken from 4 h-fasted mice and heat-inactivated (1 h at 56°C) to inactivate endogenous LPL activity. Various amounts (5, 10, 20, and 40 μl) of pooled plasma (n = 8 per group) were added to a mixture of [^{3}H]TO-labeled VLDL-like protein-free emulsion particles (0.5 mg TG/ml), apoC-II (1.25 μg/ml), bovine LPL (1 U/ml; Sigma), and FA-free BSA (60 mg/ml) in 0.1 M Tris, pH 8.5. PBS was used as a control. After 15, 30, 45, and 60 min, 20 μl of the incubation mixture was added to 1.5 ml of heptane-methanol-chloroform-oleic acid (82:115:102:0.082, v/v/v/v) and 0.5 ml of 0.2 M NaOH was added to terminate lipolysis. Generated [^{3}H]oleate was counted as described above. LPL activity was calculated by linear regression and expressed as the percentage of FA released per hour.

Statistical analysis

Statistical differences were assessed using nonparametric Mann-Whitney *U*-tests. $P < 0.05$ was regarded as significant.

RESULTS

Effect of *Hyplip2* on plasma lipid levels: fasting versus feeding

Fasted Hyplip2 mice show combined hyperlipidemia compared with B/c mice, with 26% increased plasma cholesterol (1.51 ± 0.13 vs. 1.20 ± 0.20 mM; $P = 0.004$) (**Fig. 1A**) and 64% increased plasma TG levels (1.10 ± 0.14 vs. 0.67 ± 0.10 mM; $P = 0.0002$) (Fig. 1B). In the fed state, the relative effect of Hyplip2 on plasma cholesterol levels is similar (Fig. 1A) but the difference in plasma TG levels becomes even larger (2.18 ± 0.54 and 0.84 ± 0.13 mM; $P = 0.0002$) (Fig. 1B). To investigate whether food intake contributed to these differences, we determined the intake of the regular chow diet in fully automated metabolic cages during a 2 day period. No differences in accumulated food intake were observed between Hyplip2 mice (9.8 ± 0.8 g; n = 4) and B/c

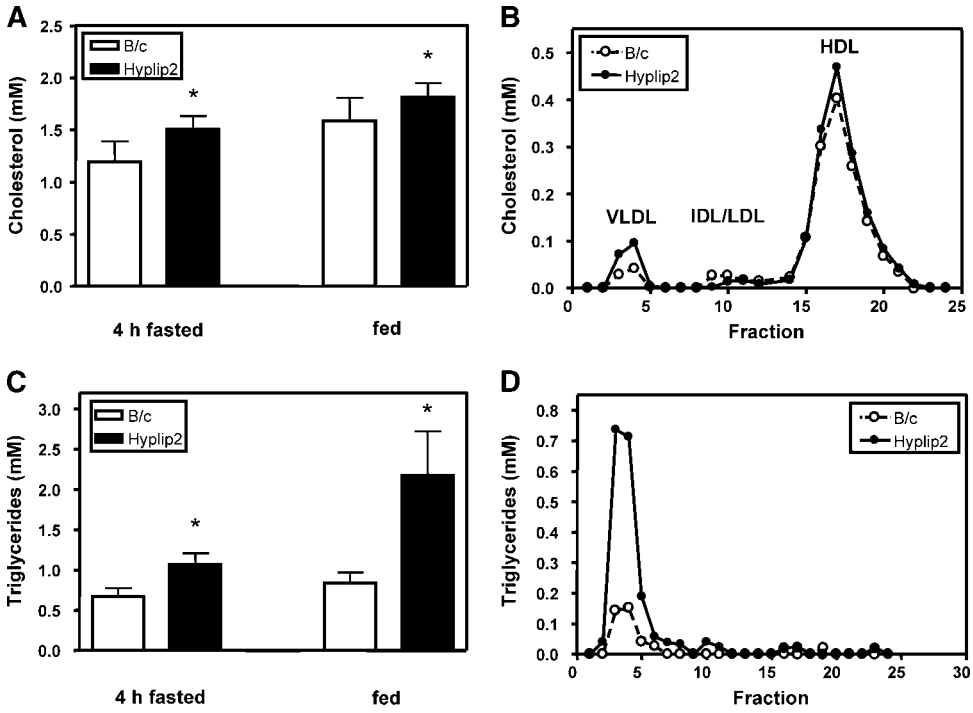

Fig. 1. Effect of *Hyplip2* locus on plasma cholesterol and triglyceride (TG) levels after 4 h of fasting and after feeding. Total cholesterol (A) and TG (C) levels are indicated. Values are means ± SD (n = 8 per group). * $P < 0.05$. Plasma of fed Hyplip2 and BALB/cJ (B/c) mice was pooled group-wise (n = 8 per group) and size-fractionated by fast-protein liquid chromatography on a Superose 6 column. The individual fractions were analyzed for cholesterol (B) and TG (D). IDL, intermediate density lipoprotein.

mice (9.5 ± 1.7 g; n = 5) ($P > 0.05$). Fractionation of lipoproteins in plasma of fed mice by fast-protein liquid chromatography showed that the increasing effect of *Hyplip2* primarily on plasma TG is attributable to a specific increase of TG-rich lipoproteins (i.e., chylomicrons and/or VLDL), whereas only a small effect was observed on HDL-cholesterol levels (Fig. 1C, D). Lipoprotein fractionation of fasted plasma yielded essentially the same results, although the effect on TG was less pronounced (data not shown).

The increased plasma TG levels in fasted and fed Hyplip2 mice can be attributable to *i*) increased hepatic VLDL-TG production, *ii*) increased intestinal lipid absorption and chylomicron production, or *iii*) decreased lipolysis and/or clearance of TG from the circulation.

Effect of *Hyplip2* on hepatic VLDL-TG production

To evaluate the effect of *Hyplip2* on hepatic VLDL-TG production, 4 h-fasted Hyplip2 and B/c mice were injected with Triton WR1339 to block lipolysis and the clearance of all TG-rich lipoproteins (11) and the accumulation of endogenous VLDL-TG in plasma were monitored over time. **Figure 2A** shows that the relative increase in TG was similar

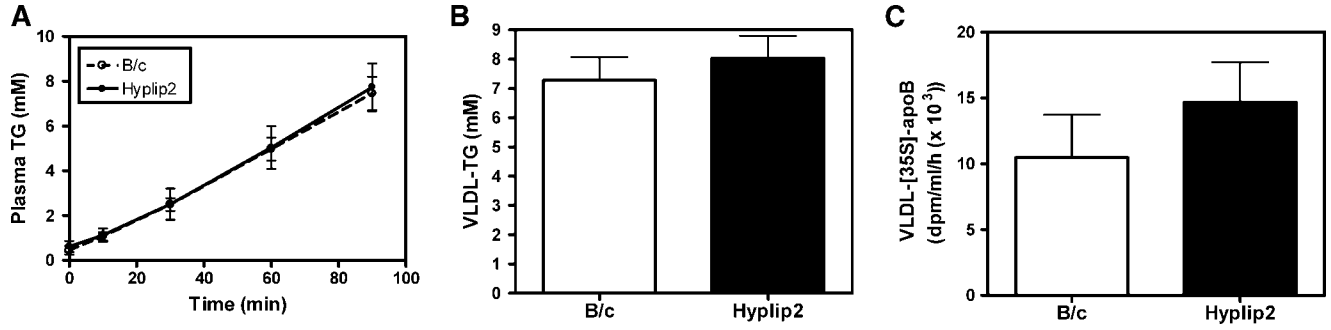

Fig. 2. Effect of *Hyplip2* locus on hepatic VLDL production. Four hour-fasted Hyplip2 and B/c mice received consecutive intravenous injections of trans-^{35}S label and Triton WR1339 (500 mg/kg body weight) to block lipolysis. Plasma samples were drawn at 10, 30, 60, and 90 min after injection and analyzed for TG (A). Values are depicted as means ± SD (n = 8 per group). After 120 min, mice were exsanguinated and VLDL was isolated and assayed for TGs (B) and [^{35}S]apolipoprotein B (apoB) (C).

in both groups of mice (4.8 ± 0.6 vs. 4.7 ± 0.4 mM TG/h; $P > 0.05$), indicating that the *Hyplip2* locus did not affect the VLDL-TG production rate. Indeed, analysis of VLDL isolated at 2 h after Triton WR1339 injection also indicated no difference in VLDL-TG content (Fig. 2B). Moreover, *Hyplip2* did not significantly affect the VLDL-[^{35}S]apoB production rate (Fig. 2C), indicating that *Hyplip2* also does not affect VLDL particle production or VLDL particle composition. The same holds true in the fed state (data not shown).

Effect of *Hyplip2* on intestinal TG absorption

Next, we investigated whether the increased plasma TG in Hyplip2 mice could be attributable to increased intestinal lipid absorption directly by intravenously injecting Triton WR1339 followed by an intragastric load of olive oil containing [^{3}H]TO. As shown in **Fig. 3**, there is no difference in the appearance of ^{3}H activity in plasma TG between Hyplip2 and B/c mice based on the area under the curve ($AUC_{0\text{-}4\ h} = 9.3 \times 10^5 \pm 6.2 \times 10^5$ and $14.7 \times 10^5 \pm 8.2 \times 10^5$ dpm × h, respectively) or the ^{3}H activity in plasma at the individual time points, indicating that *Hyplip2* does not enhance TG absorption from the intestinal lumen.

Effect of *Hyplip2* on postprandial TG response

Apparently, the increased TG levels in Hyplip2 mice cannot be explained by increased influx of TG to the plasma via increased intestinal lipid absorption or hepatic VLDL-TG production. Therefore, to gain further insight into the mechanisms underlying the hypertriglyceridemia in the Hyplip2 mouse, we determined the effect of *Hyplip2* on the postprandial TG response. To this end, 4 h-fasted mice received an intragastric bolus of olive oil (200 μl) and plasma TG levels were determined over a 6 h period (**Fig. 4**). Both groups of mice showed a postprandial increase in plasma TG, peaking at 2 h after gavage. At 1 h after gavage, the Hyplip2 mice showed 52% higher TG levels compared with the control B/c mice ($P < 0.05$). However, based on the area under the curve between 0 and 6 h ($AUC_{0\text{-}6\ h}$), there is no difference in total postprandial TG response between Hyplip2 and B/c mice ($AUC_{0\text{-}6\ h} = 2.8 \pm 1.9$ and 2.8 ± 1.8 mM × h, respectively).

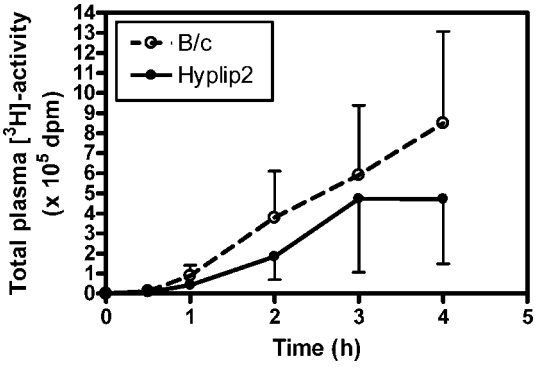

Fig. 3. Effect of *Hyplip2* locus on intestinal lipid absorption. Triton WR1339 (500 mg/kg body weight) was injected intravenously into mice that had fasted overnight. Directly after Triton injection, mice received a bolus of 200 μl of olive oil containing glycerol tri[^{3}H]oleate ([^{3}H]TO) by intragastric gavage. The total amount of plasma ^{3}H radioactivity was determined. Values represent means ± SD of eight B/c mice and seven Hyplip2 mice.

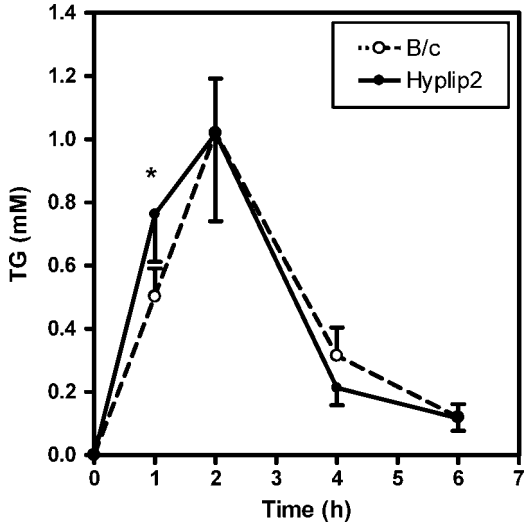

Fig. 4. Effect of *Hyplip2* locus on postprandial TG response. After 4 h of fasting, mice were given an intragastric bolus (200 μl) of olive oil. Blood samples were drawn at 0, 1, 2, 4, and 6 h after the bolus. Plasma TG concentrations were determined and corrected for plasma TG concentrations at time 0. Values represent means ± SD of 13 B/c and 9 Hyplip2 mice. * $P < 0.05$.

The fact that Hyplip2 mice show an initial increase of plasma TG levels, whereas the intestinal TG absorption is not affected, suggests that in Hyplip2 mice the lipolytic conversion of postprandial TG in plasma is impaired.

Effect of *Hyplip2* on in vivo clearance of VLDL-like emulsion particles

To investigate whether an impaired LPL-mediated TG-rich lipoprotein clearance indeed may contribute to the hypertriglyceridemia observed in Hyplip2 mice compared with B/c mice, mice were injected with [^{3}H]TO and [^{14}C]CO double-labeled protein-free VLDL-like emulsion particles, which have previously been shown to mimic the metabolic behavior of TG-rich lipoproteins (12, 16). As shown in **Fig. 5A**, the plasma clearance of [^{3}H]TO was markedly decreased in Hyplip2 mice compared with B/c mice, as evident from a 2.3-fold increased plasma half-life of [^{3}H]TO (25 ± 6 vs. 11 ± 2 min, respectively; $P < 0.05$). Concomitantly, the uptake of [^{3}H]TO-derived [^{3}H]oleate by adipose tissue was 2.2- to 7.9-fold lower in Hyplip2 mice compared with B/c mice, which reached statistical significance for gonadal adipose tissue (0.53 ± 0.24% vs. 4.18 ± 5.46% of the injected dose/g wet weight; $P < 0.05$) (Fig. 5B). The uptake of radiolabel by the liver was also 3.3-fold lower (2.12 ± 0.63% vs. 6.95 ± 1.57% of the dose/g wet weight; $P < 0.05$). These observations indicate that the TG clearance is impaired in Hyplip2 mice, which probably results from the inhibition of LPL-mediated VLDL-TG hydrolysis.

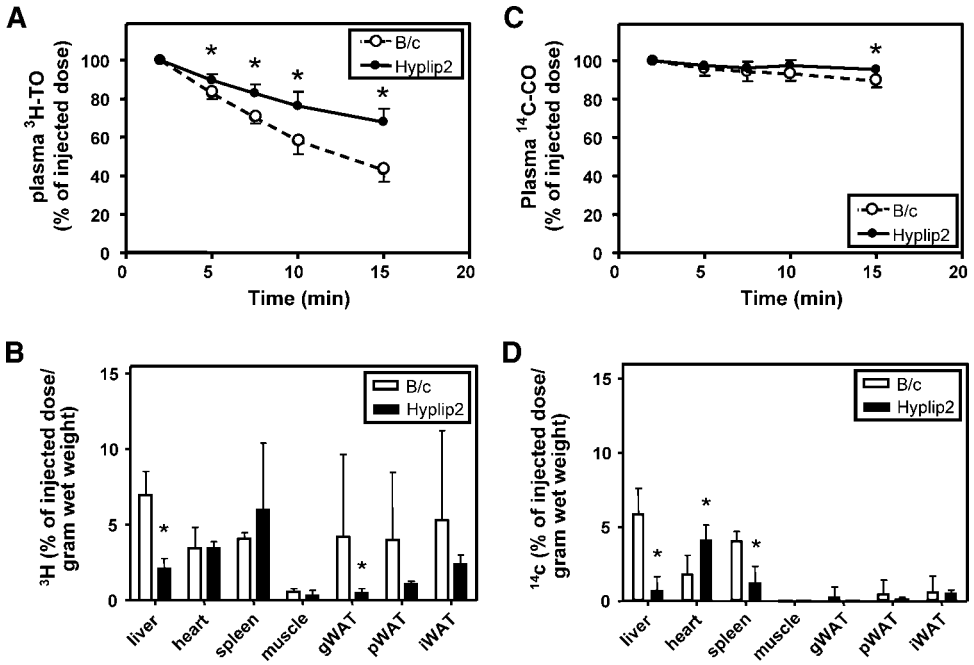

Fig. 5. Effect of *Hyplip2* locus on plasma clearance (A, C) and organ distribution (B, D) of VLDL-like emulsion particles in vivo. [^{3}H]TO and cholesteryl [^{14}C]oleate ([^{14}C]CO) double-labeled emulsion particles (1 mg of TG) were injected via the vena cava inferior into anesthetized B/c and Hyplip2 mice. Blood samples were taken at the indicated time points, and ^{3}H activity (A) and ^{14}C activity (C) were determined in plasma. After 15 min, mice were euthanized, and organs were isolated, dissolved in Soluene, and counted for ^{3}H activity (B) and ^{14}C activity (D). Values are corrected for entrapped plasma and are means ± SD (n = 4 per group). * $P < 0.05$. gWAT, iWAT, and pWAT, gonadal, intestinal, and perirenal white adipose tissue.

As a result, the plasma clearance of [^{14}C]CO was also delayed (half-life of 220 ± 78 vs. 90 ± 23 min; $P < 0.05$) (Fig. 5C) and the hepatic uptake of [^{14}C]CO (representing the particle core remnant) was 7.7-fold lower (0.77 ± 0.88% vs. 5.90 ± 1.69% of the dose/g wet weight; $P < 0.05$) (Fig. 5D) in Hyplip2 mice compared with B/c mice, respectively.

Effect of *Hyplip2* on the uptake of albumin-bound fatty acids

To evaluate whether *Hyplip2* not only inhibits LPL-mediated VLDL-TG hydrolysis but also affects the transport of FA across the endothelial layer per se, [^{3}H]TG-labeled VLDL-like emulsion particles were continuously infused together with albumin-bound [^{14}C]palmitate. Plasma half-lives of both [^{3}H]TG and [^{14}C]FA were calculated from steady-state kinetics (17). The plasma clearance of [^{3}H]TG was again delayed in Hyplip2 mice compared with B/c mice. However, the plasma half-life of [^{14}C]FA was not affected (**Table 1**). The uptake of [^{3}H]TG-derived FA by adipose tissue was 70–75% decreased in Hyplip2 mice (**Fig. 6A**). The uptake by liver (Fig. 6B), skeletal muscle (Fig. 6C), heart, spleen, and pancreas (data not shown) was unaffected. Although the uptake of TG-derived FA by the liver is lower in Hyplip2 mice in a short time frame after bolus injection of VLDL-like emulsion particles (Fig. 5B), under steady-state conditions the total liver uptake is not affected. No differences were found with respect to [^{14}C]FA uptake by adipose tissue, liver, heart, or skeletal muscle (Fig. 6). These data demonstrate that Hyplip2 does not inhibit the transport of albumin-bound FA to the adi-

TABLE 1. Effect of *Hyplip2* locus on plasma TG and fatty acid concentration, on the fractional catabolic rate of [^{14}C]FA, [^{14}C]TG, [^{3}H]FA, and [^{3}H]TG, and on the plasma half-life of [^{3}H]TG and [^{14}C]FA after continuous infusion

Strain	TG	FA	Fractional Catabolic Rate of [^{14}C]FA	Fractional Catabolic Rate of [^{14}C]TG	Fractional Catabolic Rate of [^{3}H]FA	Fractional Catabolic Rate of [^{3}H]TG	Half-Life of [^{14}C]FA	Half-Life of [^{3}H]TG
	mM			*min*$^{-1}$			*min*	
B/c	0.5 ± 0.1	1.4 ± 0.2	1.3 ± 0.7	1.4 ± 0.7	0.4 ± 0.1	0.2 ± 0.1	0.6 ± 0.3	3.7 ± 1.7
Hyplip2	1.4 ± 0.2[a]	1.4 ± 0.1	1.8 ± 0.6	0.4 ± 0.1[a]	0.4 ± 0.1	0.1 ± 0.02[a]	0.4 ± 0.1	10.7 ± 3.3[a]

B/c, BALB/cJ; TG, triglyceride. After continuous infusion of [^{3}H]TG-labeled chylomicron-like emulsion particles and albumin-bound [^{14}C]FA, blood samples were taken and the fractional catabolic rates and plasma half-lives were calculated from steady-state kinetics according to Teusink et al. (17). Values represent means ± SD of five B/c and five Hyplip2 mice.

[a] $P < 0.05$.

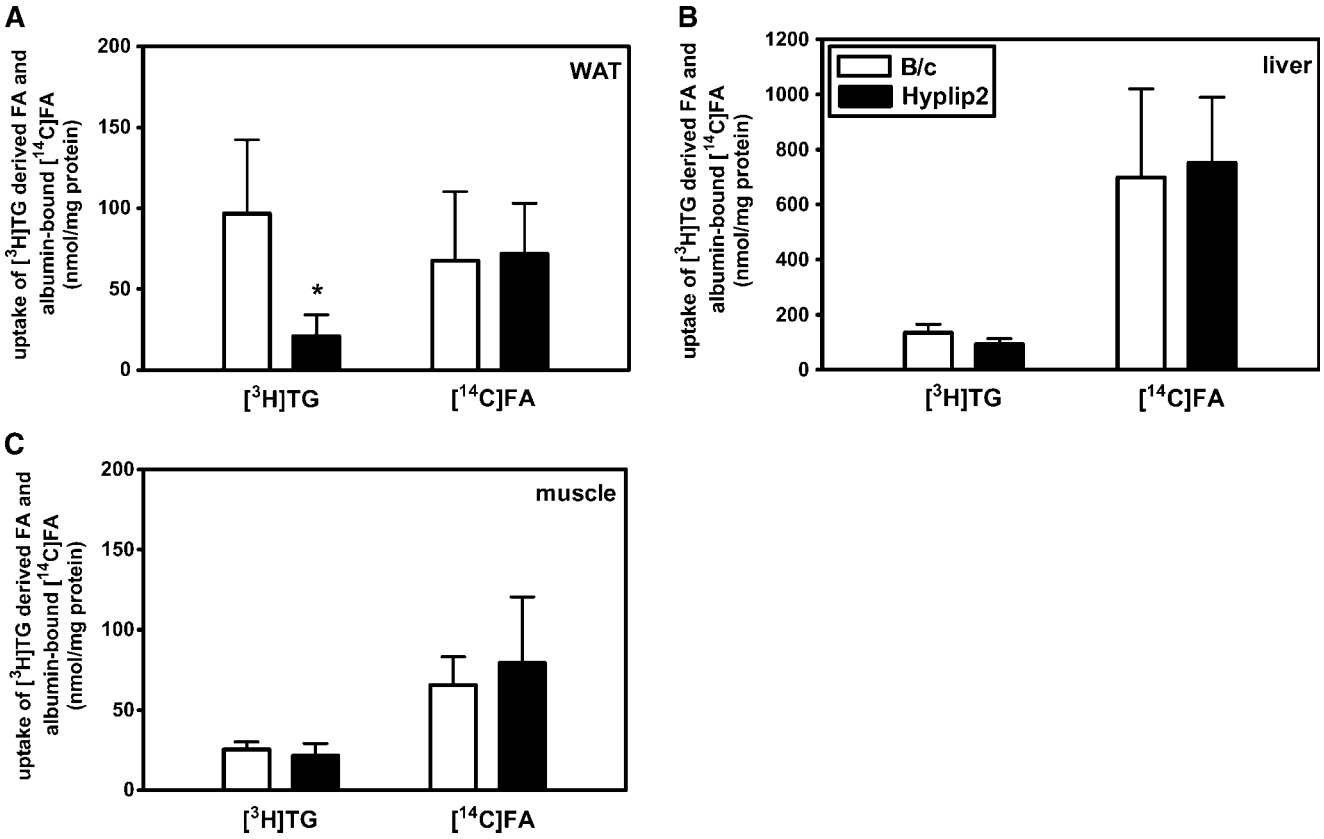

Fig. 6. Effect of *Hyplip2* locus on the uptake of TG-derived and albumin-derived FAs by the liver, muscle, and white adipose tissue (WAT). Fed B/c and Hyplip2 mice were infused for 2 h with [^{3}H]TG-labeled chylomicron-like emulsion particles and albumin-bound [^{14}C]FA. After 2 h, the mice were euthanized, and the retention of [^{3}H]TG and [^{14}C]FA was determined in white adipose tissue (A), liver (B), and muscle (C) and corrected for the specific activities of both radiolabels in plasma. Values represent means ± SD of five mice in each group. * $P < 0.05$.

pose tissue per se but selectively decreases LPL-mediated TG hydrolysis.

Effect of *Hyplip2* on plasma lipase levels

The observed delayed TG clearance in Hyplip2 mice is fully compatible with a decreased lipolytic processing of VLDL-TG and chylomicron-TG by LPL. This may be caused either by a reduction in total LPL activity present in plasma or by the modulation of LPL activity in plasma. To investigate whether Hyplip2 reduced the total available lipase activity, LPL and HL activities were measured in postheparin plasma of Hyplip2 and B/c mice. The *Hyplip2* locus did not affect the total plasma LPL or HL activity levels (**Fig. 7A**). Therefore, the impaired lipolytic conversion of VLDL in Hyplip2 mice cannot be attributable to decreased levels of LPL or HL. To investigate whether the Hyplip2 locus acts by modulation of the lipase activity rather than by reducing its expression, we studied the lipase (LPL and HL) activities in plasma in situ by allowing the interference of endogenous activators and inhibitors with lipase activity. As shown in Fig. 7B, the modulated lipolytic activity in postheparin plasma is decreased by 35% in the plasma of Hyplip2 mice compared with B/c mice (1.33 ± 0.17 vs. 2.03 ± 0.26 nmol FFA/h/ml; $P < 0.05$).

Effect of *Hyplip2* on tissue lipase activity

Although the total lipase activity in postheparin plasma is not different between B/c and Hyplip2 mice, *Hyplip2* may affect the distribution of LPL activity over the various LPL-expressing tissues. Analysis of the TG hydrolase activity in liver, heart, muscle, and white adipose tissues indeed showed that the lipase activity is increased in heart (+30%), whereas it is decreased in gonadal (−90%) and intestinal (−67%) white adipose tissue (**Fig. 8**). No differences were found in lipase activity in liver, muscle, and subcutaneous white adipose tissue. Together with a decreased modulated LPL activity in plasma of Hyplip2 mice, these results are compatible with the observed decreased uptake of [^{3}H]TG-derived FA by adipose tissue (Fig. 5).

Effect of *Hyplip2* on LPL activity in vitro

To further elucidate the presence of LPL-modifying factors in the plasma of Hyplip2 mice, we next investigated the effect of heat-inactivated plasma from Hyplip2 and B/c mice on the activity of exogenous bovine LPL. As shown in **Fig. 9**, plasma from Hyplip2 mice was less efficient at increasing bovine LPL activity (∼+25%) compared with plasma from Hyplip2 mice (∼+64%). This indicates that Hyplip2 plasma contains a higher ratio of LPL-inhibiting

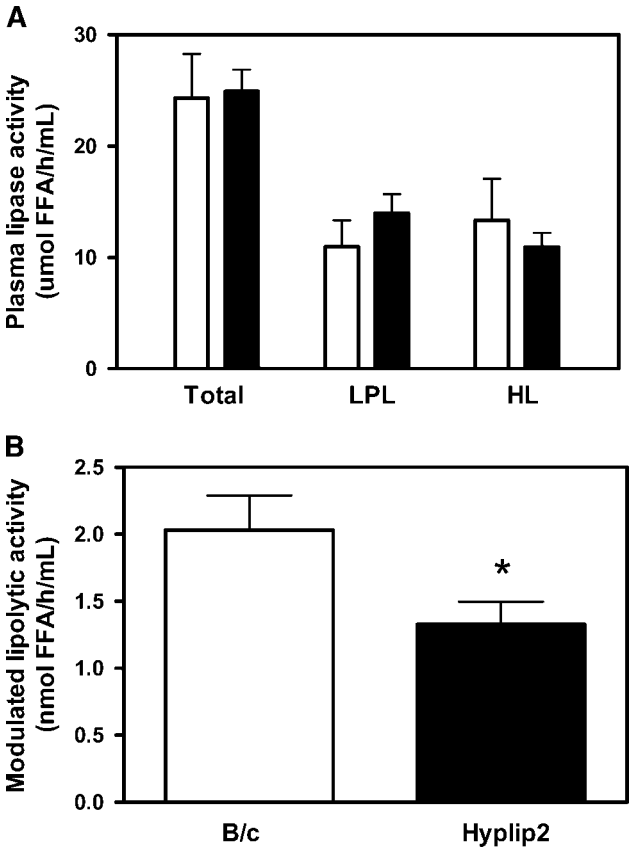

Fig. 7. Effect of *Hyplip2* locus on plasma lipase activity. Postheparin plasma was obtained after 4 h of fasting from Hyplip2 and B/c mice. A: Total plasma lipase levels were assessed by determination of [^{3}H]oleate production after incubation of plasma with a substrate mixture containing excess glycerol [^{3}H]TO and FA-free BSA as a FA acceptor. TG hydrolase activity was measured in the absence (i.e., LPL and HL) or presence (i.e., HL) of 1 M NaCl. Values represent means ± SD of seven B/c and eight Hyplip2 mice. B: The modulated lipolytic activity of postheparin plasma was assessed by incubation of plasma (1.4%) with glycerol [^{3}H]TO-labeled VLDL-like emulsion particles and excess FA-free BSA. After 1 h of incubation, samples were taken and the modulated lipolytic activity was calculated as the amount of [^{3}H]oleate produced per hour per milliliter. Values represent means ± SD of eight B/c and eight Hyplip2 mice. * $P < 0.05$.

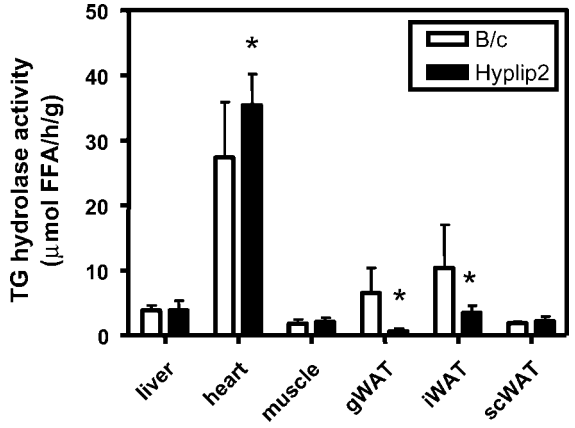

Fig. 8. Effect of *Hyplip2* locus on tissue lipase activity. The organs from 4 h-fasted Hyplip2 and B/c mice were isolated, and LPL was released by incubation in DMEM with heparin (2 U/ml). The lipase activities were assessed by determination of [^{3}H]oleate production after incubation of the supernatant with a substrate mixture containing glycerol [^{3}H]TO and FA-free BSA as a FA acceptor. Values represent means ± SD of eight mice in each group. * $P < 0.05$. gWAT, iWAT, and scWAT, gonadal, intestinal, and subcutaneous white adipose tissue.

factors than LPL-activating factors compared with plasma from B/c mice.

DISCUSSION

In a previous study, it was found that overnight-fasted Hyplip2 mice showed combined hyperlipidemia (6). In this study, we show that this combined hyperlipidemia is even more pronounced after feeding, with the most prominent increase in TG levels. We investigated the mechanism underlying this hypertriglyceridemia in Hyplip2 mice. Therefore, we studied the influence of *Hyplip2* on the metabolism of TG-rich lipoproteins: *i*) hepatic production of VLDL-TG, *ii*) intestinal TG absorption, and *iii*) TG lipolysis and uptake. The results of this study clearly show that the hypertriglyceridemia observed in Hyplip2 mice is caused by a decreased LPL-mediated TG clearance rather than by an increased influx of TG into the plasma.

To establish the effect of *Hyplip2* on the influx of TG to the plasma, we measured the absorption of lipids by the intestine and the production of VLDL-TG by the liver. Both the intestinal lipid absorption and the hepatic VLDL-TG production were not increased by *Hyplip2* and, therefore, are not the cause of the hypertriglyceridemia in the

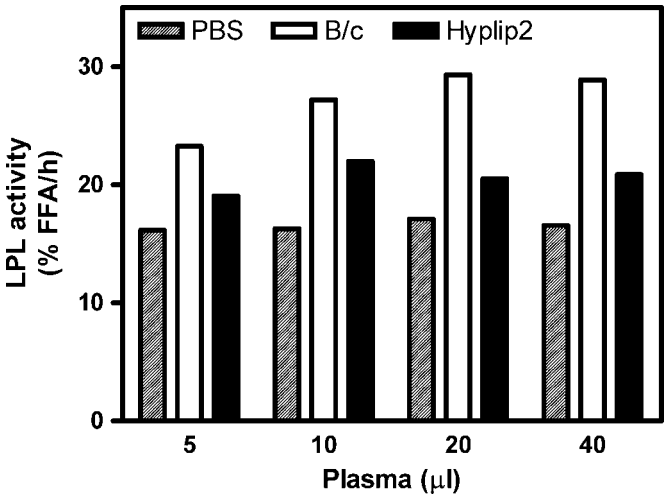

Fig. 9. Effect of *Hyplip2* locus on bovine LPL activity. Plasma was obtained from eight Hyplip2 and eight B/c mice after 4 h of fasting and pooled per group. Endogenous LPL was heat-inactivated, and 5, 10, 20, and 40 μl of plasma was added to a mixture of glycerol [^{3}H]TO-labeled VLDL-like emulsion particles, FA-free BSA, and bovine LPL. TG hydrolase activity was calculated as the percentage of [^{3}H]oleate produced per hour.

Hyplip2 mouse. In fact, we demonstrated that the 3-fold increase in plasma TG levels was attributable to the inhibition of LPL-mediated TG lipolysis. This was established by performing kinetic studies with [^{3}H]TG-labeled VLDL-like emulsion particles. After intravenous bolus injection, the plasma half-life of TG was increased and also after continuous infusion the plasma half-life of TG was increased in Hyplip2 mice compared with B/c mice in the fed state. Accordingly, the uptake of [^{3}H]TG-derived FA by adipose tissues was 62–75% decreased, whereas the uptake of albumin-bound FA was not affected, indicating that the peripheral LPL-mediated lipolysis of TG was disturbed rather than the subsequent transport of FA across the adipocyte membrane. *Hyplip2* did not affect the total lipase activity but did alter the distribution of lipase between the various tissues (i.e., increased in heart and generally decreased in white adipose tissue). This may indicate that *Hyplip2* differentially affects the translocation of LPL to the endothelium in the various tissues. The generally observed decreased lipase activity in white adipose tissue may thus contribute to the decreased uptake of [^{3}H]TG-derived FA by adipose tissues. We also observed that *Hyplip2* inhibited the modulated lipolytic activity by 35%, indicating the presence of one or more LPL-modulating factors in plasma from Hyplip2 mice. This was supported by the observation that heat-inactivated plasma of Hyplip2 mice activated exogenous bovine LPL activity to a lower extent than plasma from B/c mice. Analysis of the apolipoprotein pattern of isolated VLDL from Hyplip2 and B/c mice did not reveal any differences in protein composition (data not shown). In addition, analysis of the hepatic mRNA expression of known genes involved in LPL modulation (i.e., *ApoCI*, *ApoCII*, *ApoCIII*, *ApoAV*, *Angptl3*, and *Angptl4*) did not show any differences between Hyplip2 and B/c mice (see supplementary table).

As shown in Fig. 5, Hyplip2 mice show a decreased clearance not only of [^{3}H]TG but also of [^{14}C]cholesteryl esters, with a concomitantly reduced uptake of [^{14}C]cholesteryl esters by the liver. This implies that the increased level of cholesterol in VLDL observed in Hyplip2 mice is secondary to the hypertriglyceridemia. Apparently, the disturbance in peripheral TG hydrolysis in Hyplip2 mice is followed by a disturbance in the hepatic uptake of the remaining particle core remnants. Combined hyperlipidemia as a consequence of a disturbance in LPL function is a common phenomenon. Transgenic mice overexpressing human apoC-I (18), human apoC-III (19), or mouse apoC-III (20) also showed combined hyperlipidemia with a prominent increase in TG as a result of impaired LPL-mediated clearance of lipoproteins. In fact, direct inactivation of LPL by heterozygous disruption of LPL in *Lpl*$^{+/-}$ mice results in combined hyperlipidemia with a predominant increase of TG levels (21). Although it is thus likely that the moderate hypercholesterolemia in Hyplip2 mice is secondary to impaired LPL function, it could also be caused by different genes present in the *Hyplip2* locus that do not influence TG levels. Therefore, studying cholesterol and TG metabolism in subcongenics carrying recombinations within the congenic *Hyplip2* region will help to elucidate whether multiple causative genes are present with individual effects on plasma TG and cholesterol levels.

The molecular mechanism by which *Hyplip2* inhibits the LPL-mediated hydrolysis of TGs remains to be elucidated. It was recently shown that impairment of LPL activity can be caused by FFA-induced product inhibition (22). However, this mechanism is unlikely to contribute to higher TG levels in Hyplip2 mice, because no significant differences in plasma FFAs were found between Hyplip2 and B/c mice. However, *Hyplip2* may be involved in LPL-mediated lipolysis by influencing the interaction between lipoproteins and LPL. It may also be possible that *Hyplip2* directly interacts with LPL, thereby disturbing the active conformation and inhibiting its lipolytic function. In addition, an indirect interaction of *Hyplip2* may be involved. It may be possible that *Hyplip2* interacts with stimulators of LPL, like apoC-II (23, 24) and apoA-V (25), by displacing these apolipoproteins from TG-rich lipoproteins or by masking them. Otherwise, *Hyplip2* may also have stimulatory effects on inhibitors of LPL, like apoC-I (18, 26), apoC-III (13, 27–29), apoE (30), and the angiopoietin-like proteins angptl3 and angptl4 (31–35). Interestingly, *ApoA5* and *Angptl4* are both downstream targets of *Ppara* (36–39), which is located in the *Hyplip2* region. The fact that *Hyplip2* primarily increases TG in the fed state, whereas the effects of *Angptl4* on TG levels are most prominent in the fasted state (40), may argue against an interaction between *Hyplip2* and angptl4. Future experiments addressing the precise molecular mechanism by which *Hyplip2* increases plasma TG levels are thus warranted. This search will greatly benefit from the generation of subcongenics in which the *Hyplip2* locus has been narrowed down.

It is also intriguing to speculate about the consequences of the LPL inhibitory effects of *Hyplip2* on hepatic steatosis, obesity, and insulin resistance. The disturbed uptake of remnant particles by the liver is likely to influence the lipid content of the liver. On the other hand, the decreased uptake of VLDL-TG-derived FAs by adipose tissue may indicate that the Hyplip2 mouse would be less susceptible to diet-induced obesity and probably insulin resistance (41, 42). Because of these considerations, the role of *Hyplip2* on diet-induced obesity and insulin resistance is currently under investigation.

In conclusion, we found that the hypertriglyceridemia in the Hyplip2 mouse is caused by an impaired lipolytic conversion of VLDL particles by reduced LPL activity, resulting in a subsequent decreased delivery of TG-derived FAs to adipose tissue.

The authors thank Sigrid Fouchier for sharing data on hepatic gene expression profiles, Pjotr Prins of the Nematology Laboratory, Wageningen University, for analyzing the microarray data with microarray-quality tools, and Sjoerd van den Berg and Silvia Bijland for their help with the metabolic cages. This work was performed in the framework of the Leiden Center for Cardiovascular Research and supported by the Netherlands Heart Foundation (Grant NHS 99.150 to M.H.H and C.J.A.M.), the Netherlands Organization for Scientific Research (fellowship 903-39-174 to C.J.A.M., VIDI grant 917-36-351 to P.C.N.R. and

VENI grant 916-036-071 to P.J.V.), the Leiden University Medical Center (Gisela Thier Fellowship to P.C.N.R.), the Center of Medical Systems Biology, and the Nutrigenomics Consortium.

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