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Aspirin reduces hypertriglyceridemia by lowering VLDL-triglyceride production in mice fed a high-fat diet

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Submitted 19 April 2011; accepted in final form 22 August 2011

van Diepen JA, Vroegrijk IO, Berbée JF, Shoelson SE, Romijn JA, Havekes LM, Rensen PC, Voshol PJ. Aspirin reduces hypertriglyceridemia by lowering VLDL-triglyceride production in mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* 301: E1099–E1107, 2011. First published August 23, 2011; doi:10.1152/ajpendo.00185.2011.—Systemic inflammation is strongly involved in the pathophysiology of the metabolic syndrome, a cluster of metabolic risk factors that includes hypertriglyceridemia. Aspirin treatment lowers inflammation via inhibition of NF-κB activity but also reduces hypertriglyceridemia in humans. The aim of this study was to investigate the mechanism by which aspirin improves hypertriglyceridemia. Human apolipoprotein CI (apoCI)-expressing mice (*APOC1* mice), an animal model with elevated plasma triglyceride (TG) levels, as well as normolipidemic wild-type (WT) mice were fed a high-fat diet (HFD) and treated with aspirin. Aspirin treatment reduced hepatic NF-κB activity in HFD-fed *APOC1* and WT mice, and in addition, aspirin decreased plasma TG levels (−32%, $P < 0.05$) in hypertriglyceridemic *APOC1* mice. This TG-lowering effect could not be explained by enhanced VLDL-TG clearance, but aspirin selectively reduced hepatic production of VLDL-TG in both *APOC1* (−28%, $P < 0.05$) and WT mice (−33%, $P < 0.05$) without affecting VLDL-apoB production. Aspirin did not alter hepatic expression of genes involved in FA oxidation, lipogenesis, and VLDL production but decreased the incorporation of plasma-derived FA by the liver into VLDL-TG (−24%, $P < 0.05$), which was independent of hepatic expression of genes involved in FA uptake and transport. We conclude that aspirin improves hypertriglyceridemia by decreasing VLDL-TG production without affecting VLDL particle production. Therefore, the inhibition of inflammatory pathways by aspirin could be an interesting target for the treatment of hypertriglyceridemia.

lipid metabolism; inflammation; very low-density lipoprotein

THE METABOLIC SYNDROME IS A CLUSTERING of metabolic risk factors, including steatosis, insulin resistance, and hyperlipidemia, predisposing to the early onset of atherosclerosis and cardiovascular morbidity and mortality. It is well established that the metabolic syndrome is associated with increased systemic inflammation (28). Moreover, accumulating evidence suggests a strong involvement of systemic inflammation in the pathogenesis of components of the metabolic syndrome (4). Hypertriglyceridemia, one of the components of the metabolic syndrome and an important risk factor for the development of cardiovascular disease, is strongly associated with increased inflammation (17). Early studies show that sepsis, infection, and inflammation are accompanied by hypertriglyceridemia (8, 9, 21). More recent studies show that administration of LPS induces hypertriglyceridemia (6, 12). In addition, multiple cytokines such as IL-6 and TNFα increase serum triglyceride (TG) levels (23, 24). Therefore, inhibition of inflammation might be an attractive therapeutic target in patients with high-fat diet (HFD)-induced hypertriglyceridemia.

Nonsteroidal anti-inflammatory drugs such as aspirin are known to inhibit the enzyme cyclooxygenase. In addition, high doses of aspirin lower activation of inflammatory pathways by inhibition of the NF-κB pathway (18, 35), which plays a crucial role in the inflammation-mediated pathogenesis of the metabolic syndrome (4). Interestingly, aspirin treatment diminishes hypertriglyceridemia in both obese rodents (36) and patients with type 2 diabetes mellitus (13). However, the mechanism underlying this TG-lowering effect still has to be elucidated.

We found previously that human apolipoprotein CI (apoCI)-expressing (*APOC1*) mice have increased plasma TG by a diminished clearance of VLDL particles through apoCI-mediated inhibition of lipoprotein lipase (LPL) (2), which is aggravated by HFD feeding (Vroegrijk IO and Voshol PJ, unpublished observations). Therefore, we reasoned that the HFD-fed *APOC1* transgenic mouse is an appropriate model to study the effectiveness of treatments targeting HFD-induced hypertriglyceridemia.

The aim of this study was to investigate the mechanism by which aspirin reverses HFD-induced hypertriglyceridemia. Therefore, we studied the effect of aspirin on VLDL-TG metabolism in vivo in HFD-fed hypertriglyceridemic *APOC1* mice as well as in C57Bl/6 wild-type (WT) mice to extend any findings toward the mouse model that is most widely used for evaluation of treatments for the metabolic syndrome. Our results show that a high dose of aspirin improves hypertriglyceridemia as a consequence of a clear reduction in hepatic VLDL-TG production mediated by a diminished hepatic incorporation of plasma-derived fatty acids (FA) into VLDL-TG.

MATERIALS AND METHODS

Animals, diet, and aspirin treatment. Transgenic *APOC1* mice with hemizygous expression of the human *APOC1* gene were generated as described previously and backcrossed at least 10 times to the C57Bl/6 background. The *APOC1* mouse model develops hypertriglyceridemia mainly because of a diminished clearance of VLDL particles through apoCI-mediated inhibition of LPL (14, 16). Male *APOC1* and WT mice (also on a C57Bl/6 background) were housed under standard

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conditions with a 12:12-h light-dark cycle. At the age of 10–12 wk, mice received a HFD (45 energy% derived from palm oil, D12451; Research Diet Services, Wijk bij Duurstede, The Netherlands) for a period of 6 wk. Aspirin treatment (120 mg·kg^{-1}·day^{-1} in drinking water, pH 6.4) was given during the last 4 wk on HFD, and mice were subsequently used for experiments after an overnight fast at 9 AM. Control mice received the same drinking water of pH 6.4 without the addition of aspirin. Mice were allowed free access to food and water. Animal experiments were approved by the institutional ethics committee on animal care and experimentation at Leiden University Medical Center.

Liver NF-κB activation. Since the most common form of NF-κB is the p50/p65 heterodimer (20), the activity of both the p50 and p65 subunits in liver tissue was determined using electrophoretic mobility shift assay (22). Shortly, tissues were homogenized in ice-cold passive lysis buffer (Promega, Madison, WI) and centrifuged (14,000 rpm, 20 min; 4°C). Protein content of the supernatant was determined using the BCA protein assay kit (Pierce, Rockford, IL). For the electrophoretic mobility shift assay, the gel shift assay system was purchased from Promega. The probe was end-labeled using T4 polynucleotide kinase and [^{32}P]ATP and purified on a Microspin G-25 column (GE Healthcare, Piscataway, NJ). For each sample, 50 μg of protein was incubated with labeled probe and binding buffer (Promega) for 20 min at room temperature. Specific competition was done by adding unlabeled NF-κB binding probe to the reaction. The mixtures were run on 4.5% polyacrylamide gel electrophoresis in 0.5 × Tris-Borate-EDTA buffer. The gel was vacuum-dried and exposed to radiographic film.

Plasma parameters. Blood was collected from the tail vein into chilled paraoxon-coated capillaries (Sigma, St. Louis, MO) to prevent ongoing lipolysis (37). Capillaries were placed on ice and centrifuged, and plasma was assayed for TG, total cholesterol (TC), and phospholipids (PL) using commercially available enzymatic kits from Roche Molecular Biochemicals (Indianapolis, IN). Free fatty acids (FFA) were measured using NEFA C kit from Wako Diagnostics (Instruchemie, Delfzijl, The Netherlands). β-Hydroxybutyrate (β-HB) was determined using the enzymatic β-HB Assay kit from BioVision (Mountain View, CA)

Liver lipids. Lipids were extracted from livers according to a modified protocol from Bligh and Dyer (3). Shortly, a small piece of liver was homogenized in ice-cold methanol. After centrifugation, lipids were extracted by addition of 1,800 μl of CH_3OH-$CHCl_3$ (3:1 vol/vol) to 45 μl of homogenate. The $CHCl_3$ phase was dried and dissolved in 2% Triton X-100. Hepatic TG and TC concentrations were measured using commercial kits, as described in *Plasma parameters*. Liver lipids were expressed per milligram of protein, which was determined using the BCA protein assay kit (Pierce).

Generation of VLDL-like emulsion particles. VLDL-like TG-rich emulsion particles were prepared and characterized as described previously (26, 27). Lipids (100 mg) at a weight ratio of triolein/egg yolk phosphatidylcholine/lysophosphatidylcholine/cholesteryl oleate/cholesterol of 70:22.7:2.3:3.0:2.0, supplemented with 200 μCi of glycerol tri[9,10(n)-^{3}H]oleate ([^{3}H]TO), were sonicated at 10-μm output using a Soniprep 150 (MSE Scientific Instruments, Crawley, UK). Density gradient ultracentrifugation was used to obtain 80-nm-sized emulsion particles, which were used for subsequent experiments. TG content of the emulsions was measured as described above. Emulsions were stored at 4°C under argon and used within 7 days.

In vivo clearance of VLDL-like emulsion particles. To study in vivo clearance of the VLDL-like emulsion particles, overnight-fasted mice were anesthetized by intraperitoneal injection of acepromazine (6.25 mg/kg neurotranq; Alfasan International, Weesp, The Netherlands), midazolam (6.25 mg/kg dormicum; Roche Diagnostics, Mijdrecht, The Netherlands), and fentanyl (0.31 mg/kg; Janssen Pharmaceuticals, Tilburg, The Netherlands). Mice were injected ($t = 0$) via the tail vein with 200 μl of [^{3}H]TO-labeled emulsion particles at a dose of 100 μg TG/mouse. Blood samples were taken from the tail vein at 1, 2, 5, 10, and 15 min after injection, and plasma ^{3}H activity was counted. Plasma volumes were calculated as 0.04706 × body weight (g) as determined from ^{125}I-BSA clearance studies, as described previously (15). After the last blood sample was taken, the liver, heart, spleen, muscle, and white adipose tissue (i.e., gonadal, subcutaneous, and visceral) were collected. Organs were dissolved overnight at 60°C in Tissue Solubilizer (Amersham Biosciences, Rosendaal, The Netherlands), and ^{3}H activity was counted. Uptake of [^{3}H]TO-derived radioactivity by the organs was calculated from the ^{3}H activity in each organ divided by plasma-specific activity of [^{3}H]TG and expressed per milligram of wet tissue weight.

Hepatic VLDL-TG and VLDL-apoB production. To measure VLDL production in vivo, mice were fasted overnight, as described above. Mice were injected intravenously (iv) with Tran^{35}S label (150 μCi/mouse; MP Biomedicals, Eindhoven, The Netherlands) to label newly produced apoB. After 30 min, at $t = 0$ min, Triton WR-1339 (Sigma-Aldrich) was injected iv (0.5 mg/g body wt, 10% solution in PBS) to block serum VLDL clearance. Blood samples were drawn before ($t = 0$) and 15, 30, 60, and 90 min after injection and used for determination of plasma TG concentration, as described above. After 120 min, mice were exsanguinated via the retroorbital plexus. VLDL was isolated from serum after density gradient ultracentrifugation at $d < 1.006$ g/ml by aspiration (25) and counted for incorporated ^{35}S activity.

Hepatic gene expression analysis. Total RNA was extracted from liver tissues using the Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany) according to the instructions of the manufacturer. The quality of each mRNA sample was examined by lab-on-a-chip technology using Experion Stdsens analysis kit (Bio-Rad, Hercules, CA). One microgram of total RNA was reverse-transcribed with iScript cDNA synthesis kit (Bio-Rad), and obtained cDNA was purified with Nucleospin Extract II kit (Macherey-Nagel). Real-Time PCR was carried out on the IQ5 PCR machine (Bio-Rad) using the Sensimix SYBR Green RT-PCR mix (Quantace, London, UK). mRNA levels were normalized to mRNA levels of cyclophilin (*Cyclo*) and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). Primer sequences are listed in Table 1.

Contribution of plasma FA to VLDL-TG production. To measure the contribution of plasma-derived FA to the VLDL-TG production in vivo, mice were fasted overnight as described above. Mice received a continuous iv infusion of ^{3}H-labeled FA {[9,10(n)-^{3}H]palmitic acid in PBS with 2% bovine serum albumin} at a rate of 100 μl/h (1.6 μCi/h). After 2 h of ^{3}H-labeled FA infusion, a blood sample was taken ($t = 0$ min), and Triton WR-1339 (Sigma-Aldrich) was injected iv (0.5 mg/g body wt, 10% solution in PBS) to block serum VLDL clearance. Additional blood samples were drawn 15, 30, 60, and 90 min after injection and used for determination of ^{3}H activity in the TG fraction. Lipids were extracted by adding 10 μl of plasma to 3.25 ml of extraction fluid [heptane-methanol-chloroform, 100:128:137 (vol/vol/vol)]. [^{3}H]TGs were subsequently separated from [^{3}H]FA; 1 ml of potassium carbonate (0.1 M K_2CO_3, pH 10.5) was added, followed by vortexing and centrifugation (3,600 rpm, 15 min), leading to an upper alkaline-methanol-aqueous phase containing saponified [^{3}H]FA and a lower chloroform-organic phase containing [^{3}H]TG (1). A fraction (0.5 ml) of the total aqueous phase (2.45 ml) was counted for ^{3}H in scintillation fluid. The amount of [^{3}H]TG in each sample was calculated by distracting total [^{3}H]FA activity from total ^{3}H activity.

Statistical analysis. Data are presented as means ± SD. Statistical differences were calculated using the Mann-Whitney test for two independent samples with SPSS 16.0 (SPSS, Chicago, IL). $P < 0.05$ was regarded as statistically significant.

RESULTS

Aspirin reduces hepatic NF-κB activation. To verify that aspirin inhibits hepatic NF-κB activity, the activities of the NF-κB subunits p50 and p65 were measured in livers of *APOC1* and WT mice fed a HFD and treated with or without

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Table 1. *Primers used for quantitative real-time PCR analysis*

Gene	Forward Primer	Reverse Primer
Acox1	TATGGGATCAGCCAGAAAGG	ACAGAGCCAAGGGTCACATC
Apob	GCCCATTGTGGACAAGTTGATC	CCAGGACTTGGAGGTCTTGGA
Cd36	GCAAAGAACAGCAGCAAAATC	CAGTGAAGGCTCAAAGATGG
Cpt1a	GAGACTTCCAACGCATGACA	ATGGGTTGGGGTGATGTAGA
Cyclo	CAAATGCTGGACCAAACACAA	GCCATCCAGCCATTCAGTCT
Dgat1	TCCGTCCAGGGTGGTAGTG	TGAACAAAGAATCTTGCAGACGA
Fasn	TCCTGGGAGGAATGTAAACAGC	CACAAATTCATTCACTGCAGCC
Fabp1	GAGGAGTGCGAACTGGAGAC	GTAGACAATGTCGCCCAATG
Gapdh	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
Mttp	CTCTTGGCAGTGCTTTTTCTCT	GAGCTTGTATAGCCGCTCATT
Ppara	ATGCCAGTACTGCCGTTTTC	GGCCTTGACCTTGTTCATGT
Slc27a2	ATCGCCTATGGTATGGGACA	ACTGGCTGGCTGAGAATTTG
Slc27a4	GCTTACTCCACGGCATGACT	GTGGCTGGTTCAGGAGGTAG
Slc27a5	ATGCAGAGCTGATGATGTGG	ATCACTGTTACGCCATGCTG
Srebp1	GGAGCCATGGATTGCACATT	CCTGTCTCACCCCCAGCATA

Acox1, acyl-coenzyme A oxidase 1, palmitoyl; *Apob*, apolipoprotein B; *Cd36,* fatty acid translocase; *Cpt1a,* carnitine palmitoyltransferase 1a; *Dgat1*, diglyceride acyltransferase 1; *Fabp1*, fatty acid-binding protein 1; *Fasn*, fatty acid synthase; *Mttp*, microsomal triglyceride transfer protein; *Ppara*, peroxisome proliferator-activated receptor-α; *Slc27a2*, fatty acid transport protein 2; *Slc27a4*, fatty acid transport protein 4; *Slc27a5*, fatty acid transport protein 5; *Srebp1*, sterol-regulatory element-binding protein.

aspirin, using a gel shift assay (Fig. 1). Aspirin indeed reduced the activity of both p50 (−69%, $P < 0.05$) and p65 (−48%, $P < 0.05$) in *APOC1* mice (Fig. 1*A*) and the activity of p50 in WT mice (−72%, $P < 0.05$), whereas the reduction in the activity of p65 did not reach statistical significance ($P = 0.13$; Fig. 1*B*).

Aspirin lowers plasma TG and cholesterol levels in HFD-fed APOC1 mice. To examine whether aspirin could reduce hypertriglyceridemia in *APOC1* mice, hyperlipidemic *APOC1* mice were fed a HFD for 6 wk and treated with or without aspirin, and plasma lipids were determined (Fig. 2). Treatment of mice with aspirin reduced plasma TG levels by −32% (3.94 ± 0.15 to 2.67 ± 0.59 mmol/l, $P < 0.05$; Fig. 2*A*) and plasma TC levels by −33% (4.09 ± 0.52 to 2.76 ± 0.90 mmol/l, $P < 0.05$; Fig. 2*B*). Aspirin treatment did not affect plasma PL (Fig. 2*C*) or FFA levels (Fig. 2*D*). The reduction in plasma TG and TC levels was not caused by a reduction in body weight, since aspirin did not affect body weight in *APOC1* mice (control: 30.5 ± 2.1 g; aspirin: 28.9 ± 3.0 g). In WT mice fed a HFD for 6 wk, aspirin did not affect plasma TG, TC, PL, or FFA levels (Fig. 2, *E–H*). In addition, aspirin did not affect body weight in WT mice (control: 30.3 ± 2.1 g; aspirin: 30.8 ± 1.9 g).

Aspirin attenuates VLDL-like emulsion particle-TG clearance in HFD-fed APOC1 but not WT mice. A reduction in fasted plasma TG levels can be explained by an increase in VLDL-TG clearance and/or a decrease in hepatic VLDL-TG production. To determine whether aspirin enhances the clearance of VLDL-TG, the plasma clearance and organ distribution of [^{3}H]TO-labeled, TG-rich, VLDL-like emulsion particles was evaluated in aspirin- and control-treated hypertriglyceridemic *APOC1* mice (Fig. 3). Unexpectedly, aspirin inhibited rather than enhanced serum clearance of [^{3}H]TO ($t_{1/2}$ = 15.9 ± 6.6 vs. 5.6 ± 2.6 min; Fig. 3*A*) in *APOC1* mice. This reduction in [^{3}H]TO clearance upon aspirin was reflected by reduced uptake of [^{3}H]TO-derived radioactivity by the liver by −60% (123 ± 1 vs. 308 ± 75 nmol/g, $P < 0.05$), by skeletal muscle by −66% (11 ± 2 vs. 31 ± 15 nmol/g, $P < 0.05$), and by white adipose tissue (WAT), which reached statistical significance for gonadal WAT (12 ± 3 vs. 44 ± 22 nmol/g, $P < 0.05$) (Fig. 3*B*). Apparently, aspirin reduces rather than enhances TG clearance in *APOC1* mice and therefore cannot explain the aspirin-induced reduction in VLDL-TG. In WT mice fed a HFD for 6 wk, aspirin did not affect plasma clearance of [^{3}H]TO (Fig. 3*C*) or organ-specific uptake of [^{3}H]TO-derived radioactivity (Fig. 3*D*) in WT mice. Apparently, the decreasing effect of aspirin on TG clearance may be specific for *APOC1* mice.

Aspirin lowers VLDL-TG production in HFD-fed APOC1 and WT mice. Because the decrease in plasma TG levels in *APOC1* mice upon aspirin treatment was not caused by increased TG clearance, we investigated whether the decreased

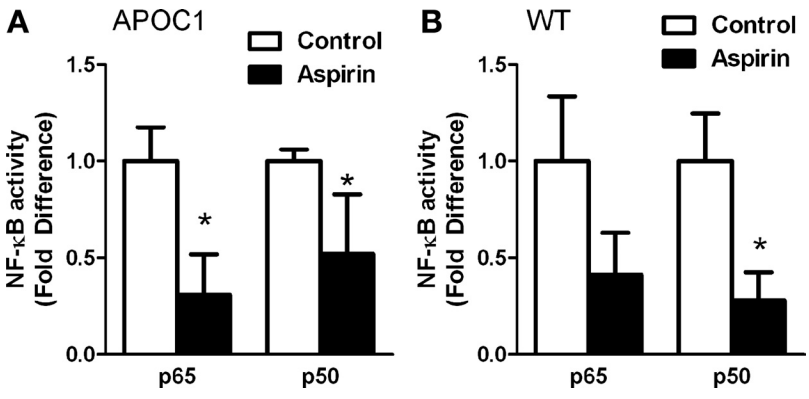

Fig. 1. Aspirin reduces hepatic NF-κB activation. Apolipoprotein (apo)CI (*APOC1*) and wild-type (WT) mice were fed a high-fat diet (HFD) for 6 wk and treated without or with aspirin. Mice were euthanized after an overnight fast, and hepatic NF-κB activity was measured by electrophoretic mobility shift assay in liver tissue of *APOC1* (*A*) and WT mice (*B*) treated without (open bars) or with aspirin (closed bars). Activities of subunits p50 and p65 were measured. Values are means ± SD (n = 3–4). *$P < 0.05$.

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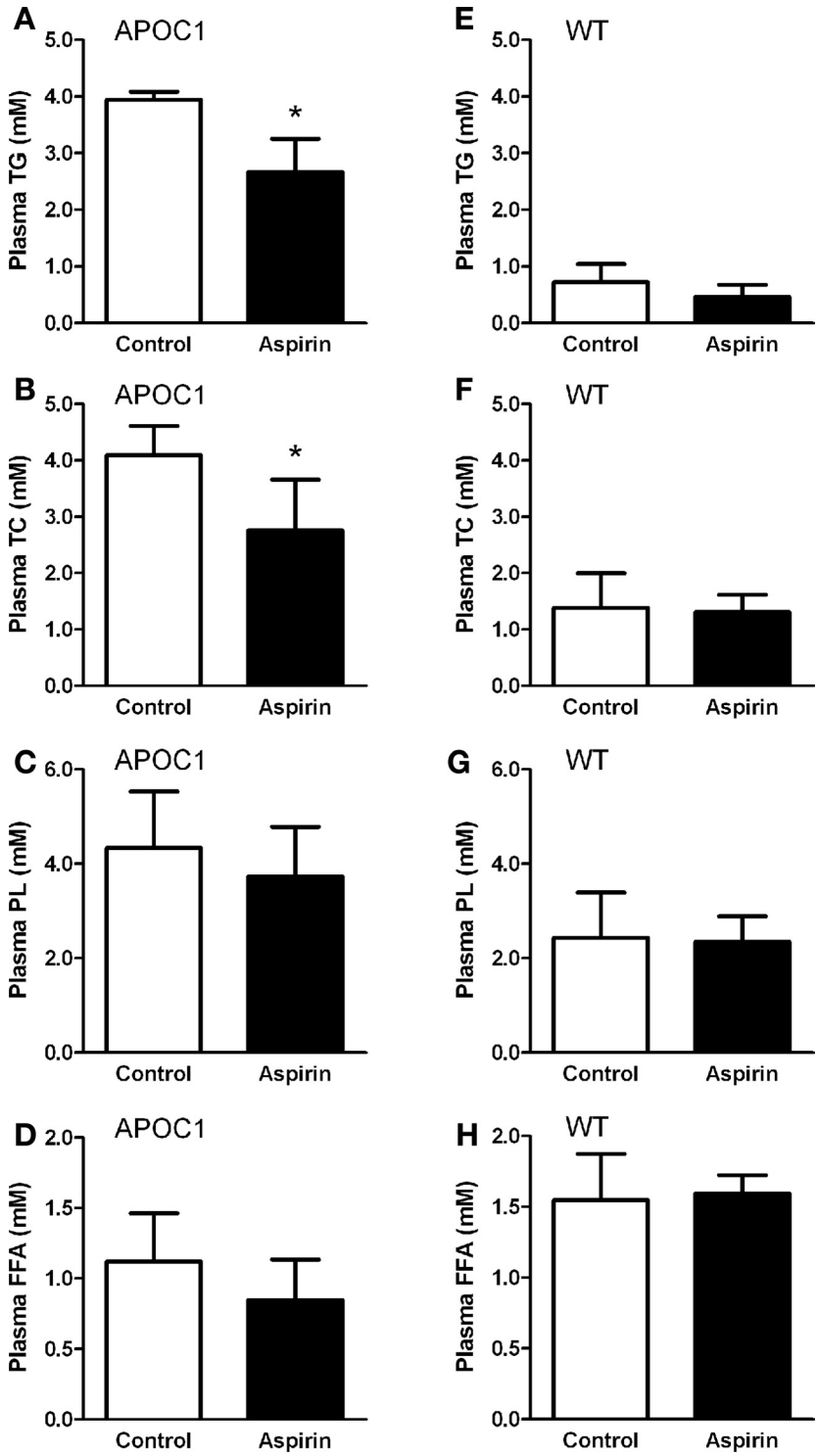

Fig. 2. Aspirin lowers plasma triglyceride (TG) and cholesterol levels in HFD-fed *APOC1* mice. Plasma TG (*A* and *E*), total cholesterol (TC; *B* and *F*), phospholipid (PL; *C* and *G*), and free fatty acid (FFA; *D* and *H*) levels were measured in plasma of overnight-fasted HFD-fed *APOC1* and WT mice treated without or with aspirin. Values are means ± SD (n = 4–5). *$P < 0.05$.

TG levels could be explained by diminished hepatic VLDL-TG production in *APOC1* mice. The rate of hepatic VLDL-TG production was measured by determining plasma TG levels after intravenous Triton WR-1339 injection (Fig. 4). We found a reduction in hepatic VLDL-TG secretion rate in *APOC1* mice treated with aspirin by −28% (3.42 ± 0.53 vs. 4.95 ± 1.11 mM/h, $P < 0.05$; Fig. 4*A*), whereas aspirin did not affect the rate of VLDL-apoB production (Fig. 4*B*). Interestingly, similar to our observation in *APOC1* mice, aspirin did reduce the hepatic VLDL-TG secretion rate in HFD-fed WT mice by −33% (2.79 ± 0.47 vs. 4.19 ± 0.48 mM/h, $P < 0.05$; Fig. 4*C*), whereas VLDL-apoB production rate was also not affected (Fig. 4*D*). Apparently, aspirin generally reduces the VLDL-TG production in HFD-fed mice independent of the genotype. Furthermore, since each VLDL particle contains a single apoB molecule, this observation shows that aspirin treatment inhibits

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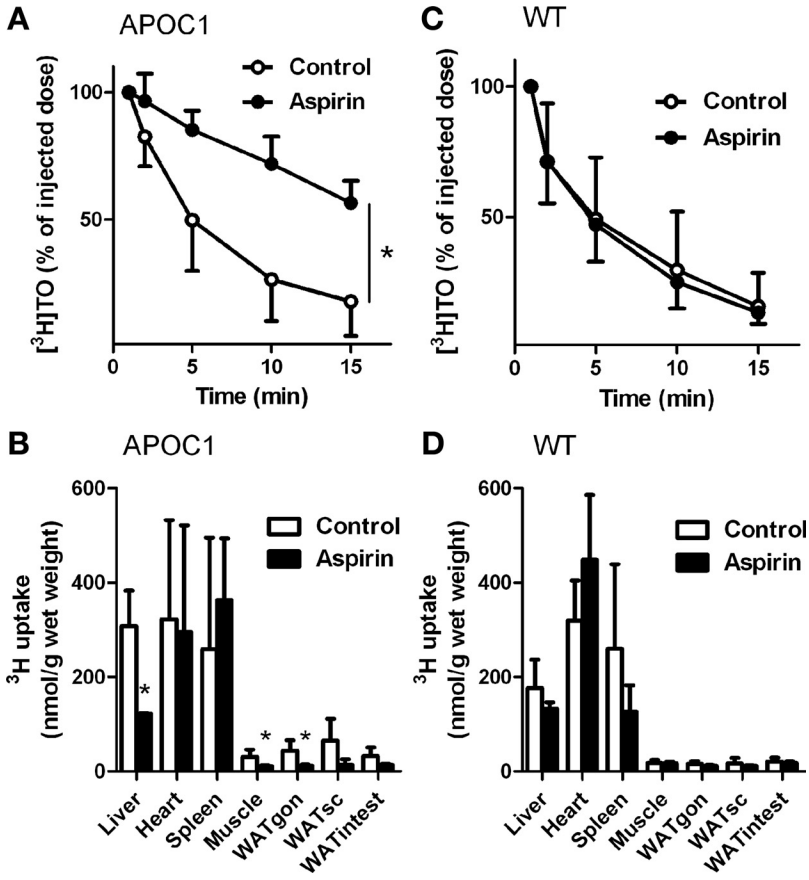

Fig. 3. Aspirin attenuates TG clearance of VLDL-like emulsion particles in HFD-fed *APOC1* but not WT mice. HFD-fed *APOC1* and WT mice that were treated without or with aspirin were fasted overnight and injected with tri[9,10(n)-^{3}H]oleate ([^{3}H]TO)-labeled VLDL-like emulsion particles. Blood was collected at the indicated time points, and radioactivity was measured in plasma of *APOC1* (*A*) and WT (*C*) mice treated without (○) or with aspirin (●). Uptake of [^{3}H]TO-derived activity by various organs was determined, and total fatty acid (FA) uptake was calculated from the specific activity of TG in plasma and expressed as nmol FA/mg wet tissue wt in *APOC1* (*B*) and WT (*D*) mice. Values are means ± SD ($n = 4$). *$P <$ 0.05. WAT, white adipose tissue; sc, subcutaneous; gon, gonadal; intest, visceral.

hepatic VLDL-TG production without affecting the rate of VLDL particle production.

Aspirin does not affect liver lipid levels in HFD-fed APOC1 and WT mice. To determine whether the attenuation in hepatic VLDL-TG production was the result of decreased lipid substrate availability in the liver, the effect of aspirin on hepatic lipid content was measured (Fig. 5). However, aspirin did not affect liver TG (Fig. 5*A*) or TC levels (Fig. 5*B*) in *APOC1* mice. Also, aspirin did not affect liver TG (Fig. 5*C*) or TC (Fig. 5*D*) levels in WT mice.

Aspirin treatment does not affect hepatic expression of genes involved in FA oxidation, lipogenesis, or VLDL production. Because changes in hepatic gene expression could underlie the reduction in VLDL-TG production, we determined the effect of aspirin on expression of genes involved in FA oxidation, lipogenesis, and VLDL production (Table 2). In both *APOC1* and WT mice, aspirin did not affect expression of peroxisome proliferator-activated receptor-α (*Ppara*), a transcription factor that regulates genes involved in FA oxidation and ketogenesis, nor did it affect its target genes acyl-coenzyme A oxidase 1 (*Acox1*) or carnitine palmitoyltransferase 1a (*Cpt1a*). In line with these results, aspirin did not increase plasma β-HB levels in WT mice (data not shown), which is a plasma marker for hepatic FA oxidation and ketogenesis. This implies that the reduced VLDL-TG production upon aspirin treatment is not caused by increased hepatic FA oxidation. We additionally determined the effect of aspirin on expression of genes involved in lipogenesis. In both APOC1 and WT mice, aspirin did not affect expression of sterol regulatory element-binding protein-1c (*Srebp-1c*), which regulates genes required for de novo lipogenesis, nor did it affect acyl/diacylglycerol transferase 1 (*Dgat1*), which catalyzes the final and only committed step in TG synthesis, or FA synthase (*Fasn*), which plays a key role in FA synthesis. These data suggest that aspirin does not affect genetic regulation of de novo lipogenesis. In addition, although aspirin induced an increase in VLDL-TG secretion, it did not affect hepatic gene expression of microsomal TG transfer protein (*Mttp*), which is involved in the assembly and secretion of VLDL. Furthermore, aspirin does not affect hepatic gene expression of apoB (*Apob*) in *APOC1* mice, which is in line with the observation that aspirin does not affect VLDL-apoB secretion in vivo. However, despite the fact that aspirin did not affect VLDL-apoB secretion in WT mice, gene expression of *Apob* was increased in WT mice.

Aspirin treatment decreases the contribution of plasma-derived FA to the VLDL-TG production. Because the decrease in VLDL-TG production was not caused by a reduced hepatic lipid content or decreased expression of genes involved in de novo lipogenesis that could reduce lipid availability for VLDL-TG secretion, we investigated whether the decreased VLDL-TG production could be explained by a diminished contribution of plasma-derived FA for VLDL-TG secretion in WT mice (Fig. 6). The contribution of plasma-derived FA was measured by determining plasma [^{3}H]TG levels after continuous [^{3}H]FA infusion and intravenous Triton WR-1339 injection. We found that aspirin reduced the hepatic [^{3}H]TG secretion rate in WT mice by −24% (3.1 ± 0.4 vs. $2.4 \pm 0.7 \times 10^3$ disintegrations·min^{-1}·h^{-1}, $P <$ 0.05), which suggests that aspirin reduces VLDL-TG production

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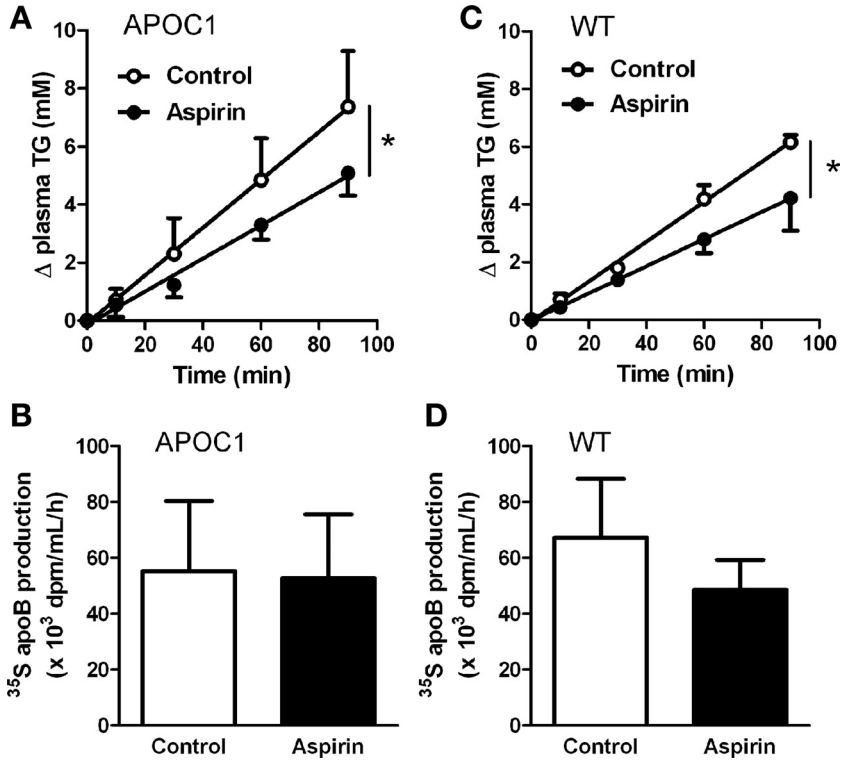

Fig. 4. Aspirin decreases VLDL-TG production in HFD-fed *APOC1* and WT mice. *APOC1* and WT mice were fed a HFD and treated without or with aspirin. Overnight-fasted mice were injected with Trans^{35}S and Triton WR-1339, and blood samples were drawn at the indicated time points. TG concentrations were determined in *APOC1* (*A*) and WT mice (*C*) treated without (○) or with aspirin (●) and plotted as the increase in plasma TG relative to $t = 0$ (*A*). After 120 min, VLDL was isolated by ultracentrifugation, ^{35}S activity was counted, and the production rate of newly synthesized VLDL-^{35}S-apoB was determined for *APOC1* (*B*) and WT (*D*). Values are means ± SD ($n = 5$). *$P < 0.05$.

by reducing the incorporation of plasma-derived FA into VLDL-TG. This reduction is not caused by a reduced hepatic expression of genes involved in hepatic FA uptake and transport (Table 2), because aspirin did not affect liver-type FA-binding protein (*Fabp1*) or FA transport proteins 2, 4, and 5 (*Slc27a2*, *Slc27a4*, and *Slc27a5*, respectively) and even increased expression of FA translocase (*Cd36*) in *APOC1* but not WT mice. These data imply that aspirin reduced the VLDL-TG production independent of changes in hepatic expression of genes involved in FA uptake and transport.

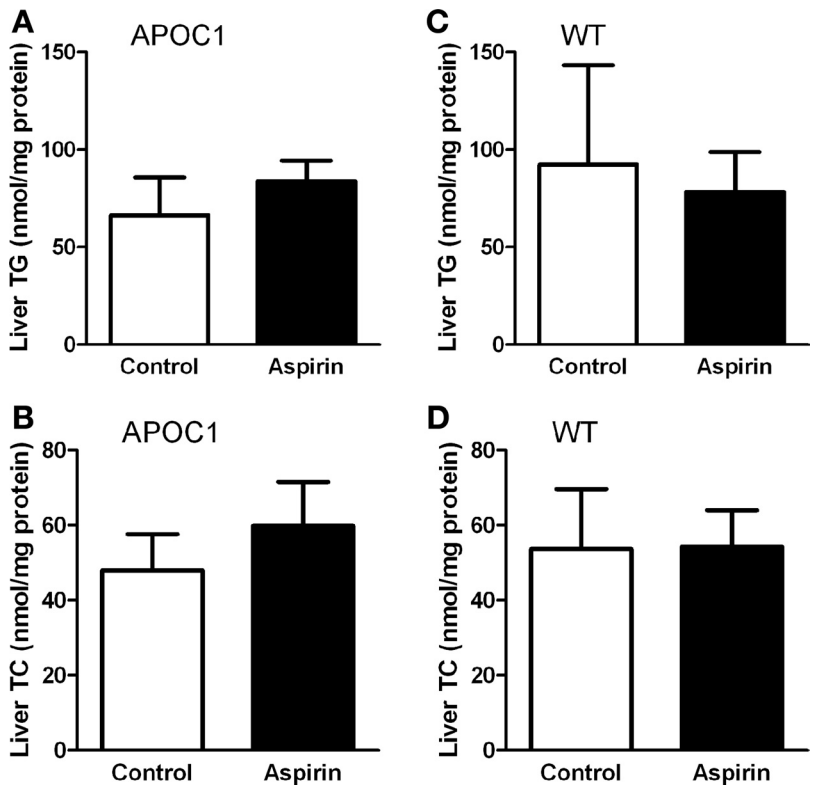

Fig. 5. Aspirin does not affect liver lipids in HFD-fed *APOC1* and WT mice. Livers were collected from overnight-fasted HFD-fed *APOC1* and WT mice treated without or with aspirin. Lipids were extracted, and TG (*A* and *C*) and TC (*B* and *D*) concentrations were measured and expressed per milligram of protein. Values are means ± SD ($n = 6$).

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Table 2. *Aspirin does not generally affect hepatic expression of genes involved in FA uptake and transport, FA oxidation, lipogenesis, or VLDL secretion*

		APOC1			WT		
Gene	Protein	Control	Aspirin	*P* Value	Control	Aspirin	*P* Value
FA uptake and transport							
Fabp1	*FABP1*	1.00 ± 0.37	0.73 ± 0.35	0.22	1.00 ± 0.41	0.61 ± 0.25	0.14
Slc27a2	*FATPa2*	1.00 ± 0.45	1.23 ± 0.49	0.46	1.00 ± 0.33	0.74 ± 0.16	0.14
Slc27a4	*FATPa4*	1.00 ± 0.35	1.74 ± 0.51	0.13	1.00 ± 0.44	1.01 ± 0.47	0.77
Slc27a5	*FATPa5*	1.00 ± 0.17	1.20 ± 0.39	0.18	1.00 ± 0.40	0.96 ± 0.31	0.62
Cd36	*CD36*	1.00 ± 0.58	1.75 ± 0.40	0.05	1.00 ± 0.80	0.45 ± 0.22	0.23
FA oxidation							
Ppara	*PPARα*	1.00 ± 0.29	1.12 ± 0.46	0.62	1.00 ± 0.37	0.72 ± 0.18	0.18
Acox1	*ACO*	1.00 ± 0.42	1.55 ± 0.55	0.14	1.00 ± 0.36	0.59 ± 0.12	0.09
Cpt1a	*CPT1a*	1.00 ± 0.55	1.36 ± 0.43	0.22	1.00 ± 0.10	0.96 ± 0.11	0.46
Lipogenesis							
Dgat1	*DGAT1*	1.00 ± 0.37	1.20 ± 0.11	0.29	1.00 ± 0.42	1.06 ± 0.53	0.85
Fasn	*FAS*	1.00 ± 0.42	1.05 ± 1.09	0.81	1.00 ± 0.40	0.97 ± 0.27	0.90
Srebp1	*SREBP-1c*	1.00 ± 0.40	1.22 ± 0.53	0.41	1.00 ± 0.53	0.85 ± 0.68	0.72
VLDL secretion							
Apob	*ApoB*	1.00 ± 0.46	1.20 ± 0.26	0.73	1.00 ± 0.32	1.59 ± 0.31*	0.03
Mttp	*MTP*	1.00 ± 0.37	0.87 ± 0.21	0.56	1.00 ± 0.39	1.21 ± 0.12	0.34

Values are means ± SD (n = 4–5). FA, fatty acid; APOC1, apolipoprotein CI; WT, wild type. Livers were isolated from overnight-fasted *APOC1* and WT mice fed a high-fat diet and treated without or with aspirin. mRNA was isolated, and mRNA expression of the indicated genes was quantified by RT-PCR. Genes are grouped as genes involved in FA uptake and transport, FA oxidation, lipogenesis, and VLDL production. Data are calculated as fold difference compared with the control group. *$P < 0.05$ compared with control group.

DISCUSSION

Treatment of obese rodents and patients with type 2 diabetes with high-dose aspirin reduces hypertriglyceridemia (13, 36). However, so far, the mechanistic basis for the relation between aspirin intake and reduced plasma TG levels has been poorly understood. In the present study we focused on the effects of aspirin on VLDL-TG metabolism in HFD-induced obese hyperlipidemic *APOC1* mice and additionally evaluated the effects of aspirin on VLDL-TG metabolism in HFD-fed normolipidemic WT mice. Our results document that aspirin treatment improves hypertriglyceridemia by reducing the hepatic production of VLDL-TG as a result of an attenuated hepatic incorporation of plasma-derived FA into VLDL-TG rather than from increased clearance of VLDL-TG from the circulation.

In the present study, aspirin treatment decreased plasma TG and TC levels in HFD-fed *APOC1* mice that display hypertriglyceridemia. This improvement in hyperlipidemia is in accord with earlier studies showing reduced serum TG concentrations upon aspirin or salicylate treatment in patients with type 2 diabetes mellitus (13) and in diabetic rats (36).

Our data show that aspirin treatment attenuated the clearance of VLDL-like TG-rich particles in *APOC1* mice. Therefore, the decrease in plasma TG levels by aspirin cannot be explained by increased TG clearance. Earlier studies report that high-dose LPS injections reduce the clearance of TG-rich lipoproteins by inhibition of the LPL activity, which is mediated by cytokines (7, 8). If indeed inflammation inhibits clearance of TG, inhibition of inflammation by aspirin is expected to increase TG-rich lipoprotein clearance, which is in contrast to our observation in *APOC1* mice. It should be noted that aspirin, in addition to inhibition of inflammation via NF-κB, also inhibits prostaglandin synthesis, which has been demonstrated to restore the LPS-induced inhibition of LPL (5). Moreover, an early report has shown that aspirin treatment inhibits postheparin LPL activity in humans (30). It would be interesting to elucidate the mechanism by which aspirin reduces the VLDL-TG clearance; however, this is beyond the scope of the present study because it does not explain the reduction in hypertriglyceridemia that we observe. Moreover, the observation may be a specific feature of the *APOC1* transgenic mouse model, since we did not observe such an effect in WT mice.

Aspirin very effectively reduced hepatic secretion of VLDL-TG in *APOC1* mice, explaining the reduction in hypertriglyceridemia upon aspirin treatment. In addition, aspirin equally reduced hepatic secretion of VLDL-TG in WT mice, indicating that the effects of aspirin on the VLDL-TG production do not exclusively occur in hypertriglyceridemic mouse models such as the *APOC1* mouse. To our knowledge, we show for the first time that a decrease in inflammation corresponds with a drop in

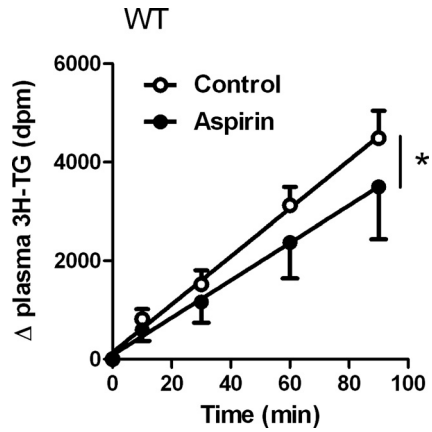

Fig. 6. Aspirin reduces the contribution of plasma-derived FA to the VLDL-TG production. WT mice were fed a HFD and treated without or with aspirin. Overnight-fasted mice received a continuous intravenous (iv) infusion of ^{3}H-labeled FA [9,10(n)-^{3}H]palmitic acid for 2 h, followed by an iv injection of Triton WR-1339. Blood samples were drawn at the indicated time points, and ^{3}H activity in the TG fraction was determined in mice treated without (○) or with aspirin (●) and plotted as the increase in plasma [^{3}H]TG relative to t = 0. Values are means ± SD (n = 7). *$P < 0.05$.

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VLDL-TG production. The reduction of VLDL-TG secretion in our study is not paralleled by a reduction in apoB secretion in either *APOC1* or WT mice, suggesting that aspirin reduces the lipidation of VLDL particles rather than the number of particles that are secreted by the liver. In contrast to our data on apoB secretion, a recent study by Tsai et al. (32) observed that suppression of IKK with BMS345541 decreased apoB secretion in vitro in primary hamster hepatocytes and HepG2 cells. Although differences between species might explain these conflicting findings, both of these published in vitro studies and our present in vivo study point toward a link between the IKK/NF-κB pathway and the regulation of VLDL production. Moreover, we have recently shown that activation of the hepatic IKK/NF-κB pathway increases VLDL-TG production (33), supporting the hypothesis that the effects of aspirin on the VLDL-TG production are mediated via a reduction in hepatic NF-κB activity. Nevertheless, activation of hepatic IKK/NF-κB increases hepatic *Fas* expression (33), whereas aspirin in the present study did not change hepatic expression of *Fasn*, nor did it change expression of other genes involved in TG synthesis, such as *Dgat1* and *Srepb-1c*, suggesting that aspirin more likely lowers VLDL-TG production by other mechanisms rather than via its effects on hepatic NF-κB activity.

A reduction in hepatic lipid availability by increased lipid oxidation could underlie the mechanism by which aspirin reduces hepatic VLDL-TG production. However, aspirin did not affect expression of genes involved in FA oxidation nor plasma levels of β-HB, a marker of hepatic FA oxidation and ketogenesis. Similarly, aspirin did not affect expression of genes involved in de novo lipogenesis or VLDL production, suggesting that aspirin does not reduce VLDL-TG production by changing expression of genes involved in hepatic lipid metabolism.

It has been suggested that the decrease in plasma TG concentration that occurs upon aspirin treatment might be secondary to the fall in plasma FFA levels (34). A reduction in FFA delivery to the liver could result in a reduced availability of FA for the release of VLDL-TG by the liver (19). Indeed, although aspirin did not change plasma FFA levels, it changed the turnover of FA, as reflected by a −24% reduction in the incorporation of plasma-derived FA into VLDL-TG, showing that aspirin in fact lowers the availability of plasma-derived FA for VLDL-TG production. This reduction of FA incorporation into VLDL-TG upon aspirin treatment was not caused by a reduced hepatic expression of FA transporter proteins, suggesting that aspirin reduces the FA incorporation via another mechanism. It is possible that aspirin reduces posttranscriptional processing of FA transporters independent of mRNA expression, since expression of FA transporters does not always correlate with changes in protein content or the rate of FA transport (10). Alternatively, aspirin might increase FA uptake and transport via simple diffusion, since FA uptake has been described independent of any FA transporter (10).

The decrease of FA turnover that we observed could be secondary to an increased insulin sensitivity of adipose tissue, thereby decreasing FA mobilization to plasma. Indeed, high-dose salicylates such as aspirin have been shown to increase insulin sensitivity (36), and the reduction in VLDL-TG production in our study is similarly accompanied by an increased insulin sensitivity (van Diepen JA and Voshol PJ, unpublished observations). However, the aspirin-induced reduction in FA utilization and subsequent VLDL-TG secretion in our study were determined under fasting conditions, when the role of insulin is marginal. In fasting conditions, the lipolytic activity of adipocytes is stimulated by catecholamines. Interestingly, aspirin has been reported to reduce catecholamine-stimulated lipolysis, which is therefore a more likely explanation for our findings (29, 31). In addition, it has been shown that aspirin reduces release of FA from adipose tissue directly via inhibition of TNFα-induced lipolysis (38). Therefore, we propose that the fact that aspirin reduces plasma-derived FA utilization by the liver is likely caused via direct inhibition of intracellular lipolysis in adipose tissue, which reduces plasma FA availability. Adipose tissue lipolysis might be further inhibited in the fed state by an increased sensitivity for insulin.

In conclusion, our data show that aspirin inhibits NF-κB and decreases HFD-induced hypertriglyceridemia by reducing hepatic VLDL-TG secretion rather than by accelerating the tissue distribution of VLDL-TG. The reduction in VLDL-TG is not caused by a decreased steatosis, increased FA oxidation, or changes in de novo lipogenesis but by an attenuation of hepatic incorporation of plasma-derived FA into VLDL-TG. In the scope of our findings, aspirin could potentially be a new therapeutic drug in the treatment of hypertriglyceridemia. However, chronic high-dose aspirin is associated with risk for bleeding. On the other hand, salsalate is a nonsteroidal, anti-inflammatory drug with similar structure that is regarded as a safer alternative. High-dose salsalate treatment has been shown recently to reduce TG levels in diabetic patients similarly to high-dose aspirin treatment (11) and could therefore potentially be a new drug for the treatment of hypertriglyceridemia.

ACKNOWLEDGMENTS

We are grateful to A. Logiantara for excellent technical assistance.

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GRANTS

This work was supported by grants from The Netherlands Organization for Scientific Research (NWO Zon-MW; 917.76.301 to P. J. Voshol) and the Dutch Diabetes Research Foundation (2005.01.003 to P. J. Voshol). I. O. C. M. Vroegrijk is supported by the Seventh Framework Program of the European Union-funded "LipidomicNet" (202272). P. C. N. Rensen is an Established Investigator of The Netherlands Heart Foundation (2009T038).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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