Overexpression of acyl-CoA synthetase-1 increases lipid deposition in hepatic (HepG2) cells and rodent liver in vivo

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Parkes, Heidi A., Elaine Preston, Donna Wilks, Mercedes Ballesteros, Lee Carpenter, Leonie Wood, Edward W. Kraegen, Stuart M. Furler, and Gregory J. Cooney. Overexpression of acyl-CoA synthetase-1 increases lipid deposition in hepatic (HepG2) cells and rodent liver in vivo. Am J Physiol Endocrinol Metab 291: E737–E744, 2006. First published May 16, 2006; doi:10.1152/ajpendo.00112.2006.—Accumulation of intracellular lipid in obesity is associated with metabolic disease in many tissues including liver. Storage of fatty acid as triglyceride (TG) requires the activation of fatty acids to long-chain acyl-CoAs (LC-CoA) by the enzyme acyl-CoA synthetase (ACSL). There are five known isoforms of ACSL (ACSL1, -3, -4, -5, -6), which vary in their tissue specificity and affinity for fatty acid substrates. To investigate the role of ACSL1 in the regulation of lipid metabolism, we used adenoviral-mediated gene transfer to overexpress ACSL1 in the human hepatoma cell-line HepG2 and in liver of rodents. Infection of HepG2 cells with the adenoviral construct AdACSL1 increased ACSL activity 10-fold compared with controls after 24 h. HepG2 cells overexpressing ACSL1 had a 40% higher triglyceride (TG) content (93 ± 3 vs. 67 ± 2 nmol/mg protein in controls, P < 0.05) after 24-h exposure to 1 mM oleate. Furthermore, ACSL1 overexpression produced a 60% increase in cellular LCA-CoA content (160 ± 6 vs. 100 ± 6 nmol/g protein in controls, P < 0.05) and increased [14C]oleate incorporation into TG without significantly altering fatty acid oxidation. In mice, AdACSL1 administration increased ACSL1 mRNA and protein more than fivefold over controls at 4 days postinfection. ACSL1 overexpression caused a twofold increase in TG content in mouse liver (39 ± 4 vs. 20 ± 2 µmol/g wet wt in controls, P < 0.05), and overexpression in rat liver increased [1-14C]palmitate clearance into liver TG. These in vitro and in vivo results suggest a pivotal role for ACSL1 in regulating TG synthesis in liver.

acetyl-coenzyme A synthetase; hepatic triglyceride synthesis; adenovirus; fatty acid metabolism

Obesity and its associated complications of insulin resistance, cardiovascular disease, hypertension, hepatic steatosis, and type 2 diabetes have become a major health problem worldwide (50). All of these metabolic disorders exhibit altered regulation of lipid metabolism, which results in the intracellular accumulation of lipid in nonadipose tissues, but the intracellular mechanisms that control partitioning of fatty acids into different metabolic pathways are still being elucidated.

Fatty acids can be metabolized in the pathways of ß-oxidation, triglyceride synthesis, phospholipid synthesis, cholesterol ester synthesis, fatty acid elongation, and protein acylation and can also serve as signaling molecules (7, 10, 40). It is believed that the partitioning of intracellular fatty acids between storage pathways and ß-oxidation is controlled by regulation of the mitochondrial acyl-CoA transporter carnitine palmitoyltransferase-1 (32), by regulation of metabolic gene expression (39), and by hormones including insulin. A number of recent studies suggest that partitioning might also involve the different isoforms of acyl-CoA synthetase (ACSL) (7, 20, 49).

ACSL catalyzes the ATP-dependent acylation of fatty acids into long-chain acyl CoAs (LCA-CoAs) and is the first step in lipid metabolism after fatty acid entry into the cell. The LCA-CoAs can then enter the ß-oxidation pathway for energy production or undergo further esterification for production of phospholipids, cholesterol esters, and triglycerides. There are five cloned isoforms of ACSL: ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 (previously known as ACS2) (48). These isoforms have a wide tissue distribution and vary in their affinity for different fatty acid substrates. ACSL1, ACSL4, and ACSL5 are all present in liver and adipocytes (22, 38, 46), whereas ACSL3 and ACSL6 are expressed in brain (12, 13). ACSL4 expression is most abundant in steroidogenic tissues and ACSL5 in intestine (22, 38).

ACSL protein appears to be localized at a number of intracellular membranes, including the plasma, microsomal, and mitochondrial outer membranes, with the active site lying on the cytoplasmic surface (3, 7, 15, 19, 29, 33, 47). By use of isoform-specific antibodies, ACSL1 was identified in the endoplasmic reticulum, mitochondria-associated membrane, and cytosol, but not in the mitochondrial fraction. ACSL4 was located predominantly in the mitochondria-associated membrane fraction and ACSL5 predominantly in the mitochondrial fraction (27).

Evidence for a role of ACSL isoforms in the channeling of fatty acids comes primarily from a number of in vitro studies using the ACSL inhibitor Triascin C. Triascin C was found to completely block de novo triglyceride synthesis in fibroblasts without inhibiting the recycling of fatty acids into phospholipid (20). In isolated rat hepatocytes, Triascin C significantly inhibited triglyceride synthesis but had little effect on oleate incorporation into cholesterol esters, phospholipids, or the end products of ß-oxidation (35). These data suggested that there are independent pools of acyl-CoAs within the cell that vary in their sensitivity to Triascin C. It was subsequently shown in a series of studies using purified ACSL1, -4, and -5 protein in vitro that Triascin C is a strong inhibitor of ACSL1 and -4 but does not inhibit ACSL5 (23). This led to the suggestion that

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ACSL1 and -4 may be preferentially linked to the triglyceride synthesis pathway of lipid metabolism, although recent studies suggest that ACSL5 may also be linked to triglyceride synthesis despite its mitochondrial location (31). Therefore, although the inhibitor studies suggest that specific ACSL isoforms may be involved in specific pathways of lipid metabolism, there is currently little direct evidence to support this hypothesis.

The aim of the current study was to investigate the role of the specific isoform ACSL1 on lipid storage and oxidation metabolic pathways by use of adenoviral-mediated gene transfer of ACSL1 (AdACSL1) in the human hepatoma HepG2 cell line and the livers of adult C57Bl/6J mice and Wistar rats.

**RESEARCH DESIGN AND METHODS**

**Generation of AdACSL1 Recombinant Adenovirus**

Recombinant adenovirus was generated and propagated according to the AdEasy protocol described by He et al. (17). cDNA for rat ACSL1 (a generous gift from Jacob Bar-Tana) was subcloned into pAdTrack-CMV and recombined into Ad5de1/E3 pAdEasy-1 to make AdGFP/ACSL1. A control adenovirus, AdGFP, was produced using the same method. A second control virus (referred to as AdEmpty) was used in the in vitro studies (5). This second control virus does not express green fluorescent protein (GFP) or any foreign cDNA. Adenovirus was purified on a CsCl gradient and then dialyzed with 10% FCS-EMEM containing 20% (wt/vol) fatty acid-to-albumin ratio within the physiological range, as given by Berk and Stump (4). Oleic acid was dissolved in ethanol (BSA) in a fatty acid-to-albumin ratio within the physiological range, immediately after the removal of medium, cells were washed four times in cold PBS and lipids extracted as described below. Incorporation of [14C]oleate into extractable neutral lipids was determined by liquid scintillation counting.

**Animal Experiments**

The study protocol for all animal experiments was reviewed and approved by the Animal Experimentation Ethics Committee (Garvan Institute of Medical Research/St. Vincent’s Hospital) and was in accordance with the National Health and Medical Research Council of Australia guidelines for animal experimentation.

**Preparation.** Male C57BL/6J mice (8–10 wk old) and male Wistar rats (10–12 wk old) were obtained from the Animal Resource Centre (Perth, Australia). Animals were housed in a temperature-controlled environment (22 ± 1°C) with a 12:12-h light-dark cycle (lights on at 0600). They were fed ad libitum on standard laboratory chow (Gordon’s Specialties Feeds, Yanderra, NSW, Australia) and had free access to water. Food intake of mice was measured every 24 h, and body weights were recorded four times in the 2 wk prior to viral infection and every day after infection. Rats were cannulated 1 wk prior to administration of adenovirus. A chronically indwelling cannula was inserted into the jugular vein under ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) anesthesia, as previously described (18). Body weight of rats was monitored daily following surgery.

**Adenoviral infection.** Mice were lightly anesthetized (methoxyflurane; Medical Developments Australia) and injected via the tail vein with 2 × 10⁹ pfu of AdGFP/ACSL1 or AdGFP, in 100 μl of vehicle, or 100 μl of 0.9% saline. Rats were administered 2 × 10¹¹ pfu of AdGFP/ACSL1 or AdGFP. Adenovirus, in 250 μl of vehicle, was infused intravenously via the jugular cannula.

**Postinfection procedures.** Mice were killed at 1, 3, 4, 5, or 7 days postinfection, a blood sample was taken from the heart, and liver and epididymal fat pads were collected and weighed. ACSL1 and GFP mRNA and protein were determined by real-time RT-PCR and Western analysis, respectively. A sample of liver tissue was rapidly frozen for analysis of total triglyceride and total LCA-CoAs as described below.

**Studies employing the tracers [9,10-³H2]-2-bromopalmitate ([H-R-BrP] and [14C]palmitate ([C-P]) were performed on anesthetized rats 4 days after adenoviral infection. Anesthesia was induced by injection of the long-acting barbiturate phenobarbitone sodium (200 mg/kg) into the indwelling jugular cannula. After cannulation of the carotid artery, rats were allowed to recover for 30 min before tracer administration, which consisted of a mixture of [³H-R-BrP (≈25 × 10⁶ dpm) and [¹⁴C]-P (≈13 × 10⁶ dpm). The [³H-R-BrP tracer was produced at AstraZeneca (Möln达尔, Sweden) (37). The [¹⁴C]-P tracer was commercially available (DuPont, Boston, MA). Tracers were delivered and blood samples collected and processed as previously described (14, 37). After collection of the final blood sample, rats were killed with an overdose (60 mg) of phenobarbitone injected via the carotid cannula. A sample of liver was rapidly frozen and stored at −70°C until analysis.

**Cell/Tissue Processing**

**Measurement of triglycerides, fatty acids, and LCA-CoAs.** Triglyceride contents of HepG2 cells, plasma, and liver were measured using an enzymatic colorimetric technique (Triglycerides GPO-PAP; Roche Diagnostics, Indianapolis, IN). Total lipids were extracted from HepG2 cells and mouse liver tissue in 2 ml of chloroform-methanol (2:1) by the method of Folch et al. (11). Phases were separated by
addition of 1 ml of 0.6% (wt/vol) NaCl, and the organic phase was evaporated under nitrogen gas. The dried lipid extract was dissolved in ethanol and used for determination of triglyceride content. Fatty acid concentration in mouse plasma and HepG2 cells medium was determined by an acyl-CoA oxidase-based colorimetric kit (WAKO FA-C; WAKO Pure Chemical Industries, Osaka, Japan). Nonesterified fatty acid (NEFA) uptake into HepG2 cells was calculated from the total NEFA concentration in the cell medium before incubation minus the NEFA concentration after incubation. Total LCA-CoAs were measured in mouse liver and HepG2 cells with a fluorometric assay, as described by Antinozzi et al. (1).

**RNA isolation and real-time RT-PCR analysis.** RNA was extracted from 100 mg of liver in 1 ml of Tri Reagent (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s instructions and quantified using absorbance at 260 nm. cDNA synthesis was performed by using a reverse transcription kit (OmniScript RT kit; Qiagen, Hilden, Germany) according to the manufacturer’s instructions, using 0.2 μg of RNA per 20 μl of reaction. Real-time PCR was performed using the LightCycler PCR machine and the LightCycler-FastStart DNA master green 1 kit (Roche). Primer sequences used for amplification were rat ACSL1: forward 5’-CAGCTGAGCATGACTATTC-3’, reverse 5’-GATCAAATCAGGATCCTG-3’; GFP: forward 5’-AAGCTGGAGTACAACTACAACAGCC-3’, reverse 5’-TTTCGGTGTTGGGTCTTTGCTC-3’; mouse GAPDH: forward 5’-TACTGCTACCAAGGCAAATT-3’, reverse 5’-ATACTACAGACCGGGCTTCAC-3’. The concentration of starting template was determined by comparison with a standard curve constructed from serial dilutions of purified ACSL1 and GAPDH PCR products. A standard curve was included in each PCR run.

**Immunoblotting procedure.** Whole cell lysate fractions were prepared from HepG2 cells by washing monolayers in ice-cold PBS and resuspending in Laemmli sample buffer (26) containing 1% SDS and 10% mercaptoethanol. Total protein was isolated from rat liver by suspending in Laemmli sample buffer (26) containing 1% SDS and 10% mercaptoethanol, mixing for 1 h at 4°C, and centrifugation at 12,000 g. Laemmli buffer was added to samples, and 10 μg of total protein were loaded per well.

Proteins were separated by SDS-PAGE on 10% gels, transferred to polyvinylidene difluoride membranes (Hybond-P; Amersham Biosciences, Buckinghamshire, UK) by electroblotting, blocked with 5% milk powder, and probed with a 1:10,000 dilution of rabbit anti-rat ACSL1 (a kind gift from Paul Watkins, Baltimore, MD) or a 1:10,000 dilution of rabbit anti-GFP (Molecular Probes, Eugene, OR). The blot was incubated with a 1:10,000 dilution of donkey anti-rabbit horseradish peroxidase-conjugated secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA), and the antigen-antibody complexes were visualized by ECL detection (PerkinElmer Life Sciences, Boston, MA) and exposure to Z-ray film (Fuji, Tokyo, Japan). Signal intensities were determined by densitometric analysis using IPLabGel software (Signal Analytics, Vienna, VA).

**Assay for ACSL activity.** HepG2 cells were seeded into six-well plates and infected with AdGFP/ACSL1 and AdGFP as described above. Cells were harvested in 100 μl of PBS/well (2 wells were pooled for each activity determination to yield ~1.6 mg of total extracted protein) and freeze-thawed to disrupt cell membranes. ACSL activity was measured in 50 μl of cell homogenate according to the method described by Ellis et al. (9).

**Plasma and Tissue Tracer Concentrations**

The method for isolation of 3H-R-BrP and 14C-P from total 3H and 14C plasma activities has been described previously (37). Tissue samples were homogenized in chloroform-methanol (2:1) with a glass-handled homogenizer. An aliquot of this homogenate was taken to determine the total activity of the 3H label. The remaining homogenate was separated into aqueous and organic phases by the addition of 1 ml of distilled water, and the 14C activity in the organic phase was used to measure the incorporation of tracer into intracellular lipid pools (18).

**Calculations**

Tissue clearance rates of 3H-R-BrP and 14C-P (Kf and Ks, respectively) were calculated from tissue content and arterial plasma profile of radiolabeled tracer and metabolic products, as previously described (37). Kf is proportional to the clearance rate of circulating NEFA, but the constant of proportionality (LC*) is not equal to 1. The results presented here have been corrected using previously determined values for LC*. Kf/LC* is an index of total tissue clearance. Ks is an estimate of the component of tissue clearance that is directed to lipid storage products. The ratio Ks/(Kf/LC*) is the fraction of cellular fatty acid uptake that is directed to lipid storage products.

**Statistical Analysis**

Statistical analyses were performed using a commercial software package (StatView: Abacus Concepts/Brainpower, Berkeley, CA). Comparisons of two groups were performed by Student’s t-test. Comparison of multiple groups was performed by one-way ANOVA incorporating a Fisher’s protected least significant difference post hoc test. The relationship between two continuous variables was examined by standard regression analysis. Group results are presented as means ± SE; P < 0.05 was considered statistically significant.

**RESULTS**

**ACSL Activity and Protein Expression in HepG2 Cells**

ACSL protein was overexpressed ~10-fold in cells infected with AdGFP/ACSL1 compared with those infected with AdGFP (Fig. 1A), and ACSL activity was increased ~20-fold in AdGFP/ACSL1-infected HepG2 cells compared with controls 36 h postinfection (Fig. 1B). There was no difference in ACSL activity in AdGFP-infected cells compared with uninfected cells. Following infection with AdGFP/ACSL1, ACSL activity in HepG2 cells increased in a linear fashion up to 24 h, at which time the activity began to plateau (Fig. 1C).

**Effect of ACSL1 Overexpression on Fatty Acid Esterification and Oxidation in HepG2 Cells**

HepG2 cells overexpressing ACSL1 had a 40% higher triglyceride content after 24 h exposure to 1.0 mM oleate compared with control cells infected with AdGFP (93 ± 3 vs. 67 ± 2 nmol/mg protein, P < 0.05). Incubation of HepG2 cells overexpressing ACSL1 in 0.5 mM [14C]oleate for 1 h increased fatty acid uptake from the medium by 43% compared with AdGFP and AdEmpty controls (data not shown). HepG2 cells infected with the AdEmpty virus had an LCA-CoA content of 100 ± 6 pmol/mg protein and converted 4.76 ± 2.08 nmol oleate to triglyceride per milligram of protein during the 1-h incubation with 0.5 mM [14C]oleate. In the same time period, the conversion of oleate to ASM was 0.53 ± 0.01 nmol/mg protein, and oleate oxidized to CO2 was 0.09 ± 0.01 nmol/mg protein. In parallel incubations, HepG2 cells infected with the AdACSL1 adenosine had a 60% increase in LCA-CoA content (Fig. 2A) and a 32% increase in [14C]oleate incorporation into extractable lipids (Fig. 2B), without significantly altering [14C]oleate conversion to CO2 or ASM (Fig. 2, C and D).
Neither ACSL1 overexpression in liver nor adenoviral infection itself had any effect on body weight of the mice at day 4 postinfection (saline-injected mice weighed 25.0 ± 0.9 g, AdGFP-injected mice 25.0 ± 0.3 g, and AdGFP/ACSL1 mice 24.6 ± 0.4 g). Similarly, there was no difference in food intake among AdGFP/ACSL1, AdGFP, and saline groups of mice compared with preinfection food intake (3.9 ± 0.2 g of chow per 24-h period per mouse).

**Liver and Fat Weight**

ACSL1 overexpression had no effect on liver weight compared with the saline-injected group at day 4 postinfection (livers of saline mice 1.10 ± 0.03 g; AdGFP mice 1.25 ± 0.03 g, and AdGFP/ACSL1 mice 1.30 ± 0.04 g, P < 0.01 for AdGFP and AdGFP/ACSL1 vs. saline). There were no differences in epididymal fat pad weight among the three groups at 4 days postinfection (0.24 ± 0.02 g in the saline group, 0.20 ± 0.02 g in the AdGFP group, and 0.23 ± 0.02 g in the AdGFP/ACSL group).

**ACSL1 mRNA and Protein Expression in Mouse Liver**

Mice were injected with AdGFP/ACSL1, AdGFP, or saline and were killed at days 1, 3, 4, 5, or 7 postinfection. Liver sections from day 1, 4, and 7 mice were examined for GFP expression via fluorescence microscopy. In the AdGFP/ACSL1 mice, GFP expression was detectable at a very low level in day 1 mice, peaked at day 4 and was significantly reduced by day 7. At day 4, GFP expression was present in 80–90% of liver cells (data not shown).

ACSL1 mRNA expression was measured using real-time RT-PCR peaked at 3–4 days post-AdGFP/ACSL1 infection, Fig. 1. Acyl-CoA synthetase (ACSL) expression and activity in HepG2 cells infected with adenovirus-administered green fluorescent protein (AdGFP)/ACSL1. A: ACSL protein in control HepG2 cells (lanes 1 and 2) or HepG2 cells 48 h postinfection with AdGFP (lanes 3 and 4) or AdGFP/ACSL1 (lanes 5 and 6). ACSL protein was measured using an anti-rat ACSL1 polyclonal antibody, as described in RESEARCH DESIGN AND METHODS. B: ACSL activity in uninfected HepG2 cells and 36 h after infection with AdGFP or AdGFP/ACSL1. ACSL was assayed as described in RESEARCH DESIGN AND METHODS. C: ACSL activity in HepG2 cells 0, 16, 24, and 48 h postinfection with AdGFP/ACSL1. Data are means ± SE; n = 3 independent experiments. ***P < 0.001 vs. AdGFP.

**Fig. 2. Effect of ACSL1 overexpression on lipid deposition and fatty acid (FA) oxidation in HepG2 cells incubated with FA.** Cells were infected with AdEmpty, AdGFP, or AdGFP/ACSL1 and incubated with 0.5 mM [14C]oleate for 1 h as described in RESEARCH DESIGN AND METHODS. A: total long-chain acyl-CoA (LCA-CoA) content of AdEmpty-, AdGFP-, or AdGFP/ACSL1-infected HepG2 cells. B: incorporation of [14C]oleate into total extractable lipid of AdEmpty-, AdGFP-, or AdGFP/ACSL1-infected HepG2 cells. C: production of [14CO2] from [14C]oleate in AdEmpty-, AdGFP-, or AdGFP/ACSL1-infected HepG2 cells. D: incorporation of [14C]oleate into acid-soluble metabolites (ASM) of AdEmpty-, AdGFP-, or AdGFP/ACSL1-infected HepG2 cells. Data are means ± SE; n = 3–5 independent experiments. ***P < 0.001 vs. AdGFP and AdEmpty controls; *P < 0.05 vs. AdGFP and AdEmpty.
where it was eightfold the level of endogenous ACSL1 expression in the AdGFP mice (Fig. 3A). There was a tendency toward increased ACSL1 mRNA in the AdGFP/ACSL1 mice killed at days 1 and 7 postinfection, but these did not reach statistical significance. ACSL protein expression was highest at days 3, 4, and 5 postinfection, where expression was three- to fourfold that of the AdGFP and saline controls (Fig. 3, B and C). To confirm that the AdGFP control and AdGFP/ACSL1 groups had comparable levels of hepatic infection, we also measured the levels of GFP mRNA and protein expression in liver in both groups of mice. There was no difference in GFP mRNA or protein expression in the liver in the two groups at day 4 postinfection, indicating that the level of adenoviral infection was similar (data not shown).

**Plasma and Liver Lipid Levels of Mice Overexpressing ACSL1**

At the time of death on day 3, 4, 5, or 7 postinfection, there was no difference in the plasma NEFA or triglyceride concentration in the AdGFP/ACSL1 or AdGFP mice compared with saline (plasma triglycerides 0.32 ± 0.03 mM; plasma NEFAs 0.54 ± 0.11 mM). The LCA-CoA content of the liver of saline-injected mice was 56.26 ± 3.42 nmol/g, and this parameter was not different in the AdGFP/ACSL1 or AdGFP mice at day 3, 4, 5, or 7 postinfection. However, ACSL1 overexpression in liver caused a twofold increase in triglyceride content at days 3, 4, and 5 postinfection (Fig. 4). Triglycerides reached a peak of 37.6 ± 3.4 nmol/g at day 4 postinfection and were back to near control levels by day 7. The transient nature of the changes in triglyceride content was similar to the observed pattern of ACSL1 overexpression. There was no difference between the liver triglyceride content of AdGFP mice and saline-injected mice (data not shown).

**ACSL and GFP Protein Expression in Rat Liver**

Four days after infection with either AdGFP/ACSL1 or AdGFP, ACSL and GFP protein content of rat liver was determined by Western blot analysis. There was no difference in the liver GFP content of AdGFP/ACSL1 and AdGFP-treated rats (Fig. 5A). In contrast (Fig. 5B), there was an approximately twofold increase (P < 0.02) in the expression of ACSL1 in the liver of rats infected with AdGFP/ACSL1.
FA uptake to storage products.

E: relationship between liver expression of ACSL protein and partitioning of (after administration of a mixture of FA tracers [9,10-3H]R-2-bromopalmitate and [14C]oleate, when livers were excised, frozen, and later analyzed for GFP protein (A), ACSL protein content by Western blot analysis (B), total FA clearance (C), and %total FA accounted for by FA storage (D). Data are means ± SE; n = 3 for AdGFP rats, n = 5 for AdGFP/ACSL1 rats. *P < 0.02.

DISCUSSION

The data from the studies reported here support the proposal that the ACSL1 isoform of acyl-CoA synthetase is linked to the storage pathway of lipid metabolism in liver and that it may act to channel fatty acids into triglyceride synthesis rather than into β-oxidation and energy production. Overexpression of ACSL1 in HepG2 hepatoma cells by use of adenoviral transfer caused a 20-fold increase in ACSL activity compared with cells infected with the AdGFP control. When incubated for 1 h with 0.5 mM oleate, AdACSL1-infected HepG2 cells showed a significant increase in fatty acid uptake, LCA-CoA content, and incorporation of [14C]oleate into glycerolipids, without a significant change in indexes of β-oxidation (Fig. 2).

The in vivo relevance of this data is strongly supported by our studies overexpressing ACSL1 in mouse and rat liver. In mouse liver, ACSL1 overexpression produced a twofold increase in the triglyceride content of the liver 3, 4, and 5 days postinfection, which coincided with the peak of ACSL1 gene and ACSL protein expression. This increase in triglyceride content in liver of adult mice fed a normal low-fat diet after just 2–3 days of ACSL1 overexpression was similar to that found after 3 wk of high-fat feeding in mice (34). ACSL1 overexpression had no effect on food intake or body weight, suggesting that any changes in lipid metabolism were the result of changes in the regulation of lipid metabolism in the liver. Moreover, the in vivo tracer studies in rats demonstrated that ACSL1 overexpression appeared to specifically partition intracellular fatty acid toward storage products rather than increase the ability of the liver to clear circulating fatty acids. Thus, the in vitro and in vivo data indicate that ACSL1 can play a significant role in the regulation of the flux through the lipid synthesis pathway in hepatocytes.

The mechanism by which ACSL1 facilitates partitioning of fatty acids to lipid synthesis remains unclear. It has been suggested that the subcellular location of ACSL isoforms is an important indicator of their suggested role in fatty acid partitioning. ACSL1 is reportedly associated with endoplasmic reticulum, plasma membrane, GLUT4 vesicles, and cytosol rather than mitochondria and therefore more linked to the synthetic pathways of fatty acid metabolism (15, 27, 44). Because of its association with mitochondrial membranes, ACSL5 was thought to be associated with fatty acid oxidation; however, recent studies using adenoviral expression in McArdle-RH7777 cells suggest that overexpression of ACSL5, like ACSL1, also facilitates fatty acid incorporation into triglyceride. One possible explanation for the similar effect of overexpressing ACSL1 and ACSL5 in cultured hepatic cells is that an increase in any of the isoforms of ACSL would increase LCA-CoA concentration and trap fatty acids inside the cell. Because the cells do not have a metabolic need to oxidize the fatty acids for energy, the LCA-CoAs would be esterified to triglyceride. Therefore, the overall energy status of the cell could also influence partitioning rather than just the relative abundance of various ACSL isoforms.

How ACSL isoforms are localized to distinct organelles is not obvious, because there appears to be no molecular, catalytic, or immunologic differences in an isoform present at
various subcellular sites (15, 33, 47). Work with adipocytes has suggested that changes in ACSL activity at the various subcellular locations may be more important for determining fatty acid partitioning than translocation or increases in the total amount of ACSL protein (49). It is also possible that acylation, phosphorylation, or association of the various isoforms of ACSL alter their activity or specificity to influence partitioning irrespective of the subcellular location of the isoforms. Whatever the mechanism, our results clearly demonstrate that increased amounts of ACSL1 in rodent liver increase the partitioning of fatty acids to triglyceride storage.

In the in vivo studies, adenovirus was administered intravenously with the aim of obtaining liver-specific overexpression in adult C57BL/6J mice and Wistar rats. Several reports in the literature have demonstrated that infection of adenovirus into the tail vein of mice results in >99% of the virus infecting liver (2, 25), and as expected, we did not observe GFP expression in any other tissue, including lung, spleen, pancreas, skeletal muscle, or heart.

In HepG2 cells, adenoviral infection resulted in a >10-fold increase in ACSL activity, but the increase in fatty acid uptake and conversion to triglyceride was of a lesser magnitude. The accumulation of LCA-CoAs observed in HepG2 cells overexpressing ACSL1 is consistent with the proposal that enzymes other than ACSL1, such as glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT), are limiting to triglyceride synthesis in HepG2 cells under these experimental conditions. In vivo, the fivefold overexpression of ACSL1 following AdACSL1 infection in liver increased triglyceride content twofold without any significant change in LCA-CoA content, possibly indicating a greater capacity for conversion of LCA-CoA to triglycerides in vivo compared with the cell culture system. Therefore, although ACSL1 activity appears to be an important determinant of triglyceride deposition, other aspects of fatty acid metabolism pathways would also contribute (16, 28, 45).

Other evidence exists to suggest that ACSL, and more specifically ACSL1, is an important factor in tissue lipid accumulation. ACSL activity is increased in red gastrocnemius muscle of high-fat-fed rats compared with lean controls and accumulation. ACSL activity is increased in red gastrocnemius muscle, or heart.

Increased lipid deposition.

In HepG2 cells, adenoviral infection resulted in a >10-fold increase in ACSL activity, but the increase in fatty acid uptake and conversion to triglyceride was of a lesser magnitude. The accumulation of LCA-CoAs observed in HepG2 cells overexpressing ACSL1 is consistent with the proposal that enzymes other than ACSL1, such as glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT), are limiting to triglyceride synthesis in HepG2 cells under these experimental conditions. In vivo, the fivefold overexpression of ACSL1 following AdACSL1 infection in liver increased triglyceride content twofold without any significant change in LCA-CoA content, possibly indicating a greater capacity for conversion of LCA-CoA to triglycerides in vivo compared with the cell culture system. Therefore, although ACSL1 activity appears to be an important determinant of triglyceride deposition, other aspects of fatty acid metabolism pathways would also contribute (16, 28, 45).

Other evidence exists to suggest that ACSL, and more specifically ACSL1, is an important factor in tissue lipid accumulation. ACSL activity is increased in red gastrocnemius muscle of high-fat-fed rats compared with lean controls and may contribute to the accumulation of intramuscular lipid in this model (18). In the Zucker obese rat model, ACSL activity and mRNA were found to be significantly increased in the liver and adipose tissues compared with their lean littermate controls (42). Furthermore, ACSL mRNA was significantly reduced in the intra-abdominal visceral fat of chow-fed Wistar rats after a period of exercise (43). Transgenic overexpression of ACSL1 specifically in mouse heart resulted in lipid accumulation and cardiomyopathy in cardiac myocytes, demonstrating that ACSL1 is a key enzyme in lipid metabolic pathways in heart (6). The recent report of ACSL5 overexpression in rat hepatoma McArdle-RH-7777 cells resulting in preferential metabolism of fatty acids to triglycerides again emphasises the potential importance of ACSL enzymes in regulating the metabolic fate of fatty acids in hepatocytes, although these data using ACSL5 suggest that an increased capacity of ACSL rather than an increase in a specific isoform may be more relevant to channeling fatty acids to triglycerides (31).

The liver is the regulation to the control of whole body lipid homeostasis. It plays an important role in regulating the level of circulating lipids by altering the rates of liver NEFA uptake, fatty acid synthesis, VLDL secretion, and fatty acid oxidation. Hepatic steatosis is commonly associated with obesity and insulin resistance, and in some people steatosis can progress to nonalcoholic steatohepatitis (NASH), cirrhosis, and liver failure (8, 24, 30, 41). Inappropriate hepatic lipid deposition can result from a number of causes, including overnutrition, poor nutrition, or infections. However, a recent study in a large cohort of Japanese subjects found an association between a polymorphism in the acyl-CoA synthetase gene SAH, and hypertriglyceridemia, hypercholesterolemia, obesity, and hypertension highlight the possible importance of ACSL gene expression in metabolic disease (21).

The studies reported here are consistent with the idea that changes in ACSL1 in vivo could channel fatty acids into triglyceride synthesis rather than into β-oxidation for energy production. Although our data represent the first report of the effect of overexpressing ACSL1 in liver in vivo, comparison of the effects of expressing other isoforms of ACSL in vivo are needed to conclusively determine the role of specific ACSL isoforms in the partitioning of fatty acids. Nevertheless, our findings, together with other reports in the literature, suggest that altered expression of ACSL, and ACSL1 in particular, may have implications for the accumulation of triglyceride in liver and the subsequent metabolic problems associated with increased lipid deposition.

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