Original article

MOLECULAR METABOLISM

Acyl CoA synthetase 5 (ACSL5) ablation in mice increases energy expenditure and insulin sensitivity and delays fat absorption

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ABSTRACT

Objective: The family of acyl-CoA synthetase enzymes (ACSL) activates fatty acids within cells to generate long chain fatty acyl CoA (FACoA). The differing metabolic fates of FACoAs such as incorporation into neutral lipids, phospholipids, and oxidation pathways are differentially regulated by the ACSL isoforms. *In vitro* studies have suggested a role for ACSL5 in triglyceride synthesis; however, we have limited understanding of the *in vivo* actions of this ACSL isoform.

Methods: To elucidate the *in vivo* actions of ACSL5 we generated a line of mice in which ACSL5 expression was ablated in all tissues ($ACSL5^{-/-}$).

Results: Ablation of ACSL5 reduced ACSL activity by ~80% in jejunal mucosa, ~50% in liver, and ~37% in brown adipose tissue lysates. Body composition studies revealed that $ACSL5^{-/-}$, as compared to control $ACSL5^{loxP/loxP}$, mice had significantly reduced fat mass and adipose fat pad weights. Indirect calorimetry studies demonstrated that $ACSL5^{-/-}$ had increased metabolic rates, and in the dark phase, increased respiratory quotient. In $ACSL5^{-/-}$ mice, fasting glucose and serum triglyceride were reduced; and insulin sensitivity was improved during an insulin tolerance test. Both hepatic mRNA (~16-fold) and serum levels of fibroblast growth factor 21 (FGF21) (~13-fold) were increased in $ACSL5^{-/-}$ as compared to $ACSL5^{loxP/loxP}$. Consistent with increased FGF21 serum levels, uncoupling protein-1 gene (*Ucp1*) and PPAR-gamma coactivator 1-alpha gene (*Pgc1α*) transcript levels were increased in gonadal adipose tissue. To further evaluate ACSL5 function in intestine, mice were gavaged with an olive oil bolus; and the rate of triglyceride appearance in serum was found to be delayed in $ACSL5^{-/-}$ mice as compared to control mice.

Conclusions: In summary, $ACSL5^{-/-}$ mice have increased hepatic and serum FGF21 levels, reduced adiposity, improved insulin sensitivity, increased energy expenditure and delayed triglyceride absorption. These studies suggest that ACSL5 is an important regulator of whole-body energy metabolism and ablation of ACSL5 may antagonize the development of obesity and insulin resistance.

Keywords Dietary fat absorption; Acyl-CoA; ACSL; Intestine; Liver; FGF21

1. INTRODUCTION

Alterations in fatty acid trafficking and metabolism have been implicated as critical factors in the development of obesity and its associated complications such as non-alcoholic fatty liver disease (NAFLD), and type2 diabetes (T2DM) [1–4]. Central to our understanding of fatty acid trafficking are proteins that determine the metabolic fate of fatty acids. The first step in intracellular metabolism of fatty acids is the addition of a CoA group catalyzed by the actions of long-chain acyl-CoA synthetases (ACSL) [5,6]. Five related ACSL isoforms have been delineated in rats, mice and humans [5,7]. It has been hypothesized that the different ACSL isoforms may direct fatty acids to different metabolic fates depending upon the protein's subcellular localization and tissue specific expression [6].

At the present time, we have limited understanding about the relative contributions of acyl CoA synthetase 5 (ACSL5) to *in vivo* lipid

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Abbreviations: ACSL, long-chain acyl-CoA synthetase; ACSL5−/−, mice with global ablation of ACSL5; FGF21, fibroblast growth factor 21; UCP1, uncoupling protein-1; PPAR, peroxisome proliferator activated receptor; PGC1α, PPAR-gamma coactivator 1α; NAFLD, non-alcoholic fatty liver disease; T2DM, type2 diabetes; SREBP1c, steroid response element binding protein-1c; VLDL, very low density lipoprotein; ES, embryonic stem; ITT, insulin tolerance test; SDS, sodium dodecyl sulfate; AUC, area under the curve; RER, respiratory exchange ratio

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metabolism. ACSL5 has been reported to be highly expressed in liver, small intestine, white preadipocytes, and brown adipose tissue [7,8]. Hormonal regulation of hepatic ACSL5 expression was suggested by the observation that both fasting and streptozocin-induced diabetes reduced ACSL5 expression, while carbohydrate feeding increased the protein's expression [7–9]. These prior observations indicated that insulin and its downstream mediators such as the nuclear transcription factor, SREBP-1C, regulate ACSL5 expression [10,11]. A role for SREBP1c in regulating ACSL5 expression was demonstrated when adenoviral mediated overexpression of SREBP1c in rats with streptozocin-induced diabetes rescued hepatic ACSL5 expression [9,10].

Overexpression of ACSL5 in rat hepatoma McCardle-RH777 cells increased both fatty acid uptake and conversion of fatty acids to triacylglycerol [7]. Additionally, siRNA mediated knockdown of ACSL5 in isolated rat hepatocytes reduced triglyceride accumulation and VLDL secretion while increasing fat oxidation [12]. Furthermore, studies of rodents have demonstrated that ACSL5 is most highly expressed within jejunal enterocytes [8], which alludes to the possibility that ACSL5 may be involved in fat absorption during the reesterification of dietary fatty acids into triglyceride. In jejunal enterocytes, triglyceride is packaged and secreted as chylomicrons that ultimately circulate in the blood. Importantly, ACSL5 was found to be present on cytoplasmic lipid droplets in jejunal enterocytes [13]. However, at the present time we have little understanding of ACSL5's role in intestinal lipid metabolism.

As a first step to elucidating the *in vivo* role of ACSL5 in tissue specific and systemic metabolism, our laboratory has generated mice in which ACSL5 expression is ablated in all tissues ($ACSL5^{-/-}$). Investigations of these mice demonstrate that loss of ACSL5 expression significantly reduced total ACSL activity in liver, brown adipose tissue, and most robustly within jejunum. Intriguingly, ablation of ACSL5 resulted in increased hepatic mRNA and serum FGF21 levels in mice consuming a chow diet. Consistent with increased FGF21 expression, $ACSL5^{-/-}$ mice have increased metabolic rate, reduced fat mass and serum TG, improved insulin sensitivity, and increased expression of UCP1 in white adipose tissue depots. Interestingly, triglyceride absorption studies demonstrated a role for ACSL5 in dietary fat absorption.

2. MATERIALS AND METHODS

2.1. Generation of ACSL5 deficient mice ($ACSL5^{-/-}$)

We collaborated with Genoway (Lyon, France) to generate a line of conditional ACSL5 knockout mice. The ACSL5 gene was isolated from a C57BL6/J library and loxP sequences inserted in introns flanking exons 16 and 17 (exons nomenclature based on NM_027976.2 sequence). The ACSL5 gene construct was introduced in C57BL6/J embryonic stem (ES) cells; ES cells were selected for homologous recombination, injected into mice, and progeny were mated to generate line of mice in which the ACSL5 gene was floxed ($ACSL5^{loxP/loxP}$) (See Figure 1). Selection of recombined construct in C57BL6/J ES

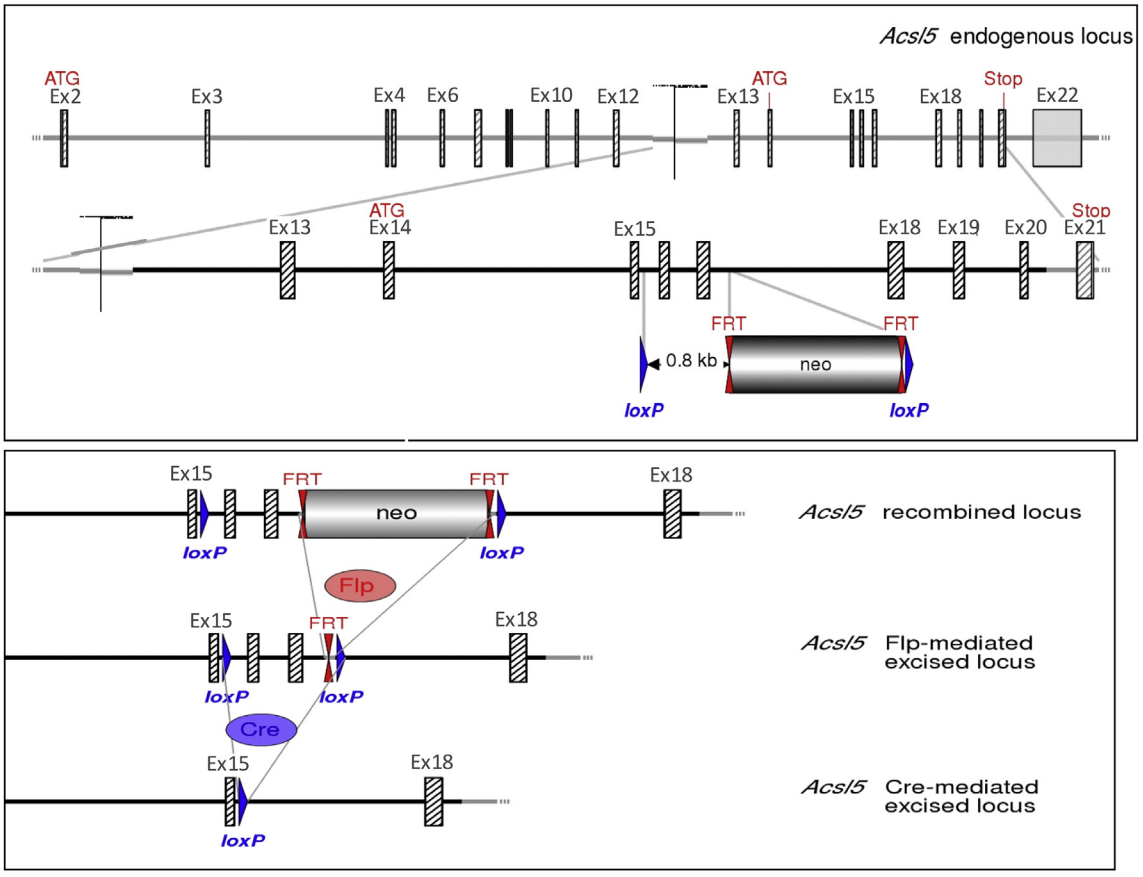

Figure 1: Schematic representation of *Acsl5* targeting strategy, resulting in deletion of exons 16–17. Diagram is not depicted to scale. Hatched rectangles represent Acsl5 coding sequences, gray rectangles indicate non-coding exon portions, and solid lines represent chromosome sequences. In upper panel, the initiation (ATG) and Stop (Stop) codons are indicated. FRT sites are represented by double red triangles and loxP sites by blue triangles. The size of the flanked Acsl5 sequence to be deleted is shown. The strategy results in the deletion of 206 bp of coding sequences encoding for part of the AMP binding domain. The splicing of exon 15 to exon 18 will lead to a frame shift resulting in a premature stop codon in exon 20. In lower panel, the scheme of Cre recombinase- or Flp recombinase-mediated excision at the recombined Acsl5 locus.

