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# Acetoacetyl-CoA synthetase gene is abundant in rat adipose, and related with fatty acid synthesis in mature adipocytes

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**Abstract**

Acetoacetyl-CoA synthetase (AACS, acetoacetate-CoA ligase, EC 6.2.1.16) is a novel cytosolic ketone body (acetoacetate)-specific ligase, the physiological role of which remains to be elucidated. We examined the expression profiles of AACS mRNA in adult rat tissues, finding that it was particularly abundant in male subcutaneous white adipose tissue after weaning. In white adipose tissue, AACS mRNA was preferentially detected in mature adipocytes but not in preadipocytes. The AACS mRNA expression in primary preadipocytes increased during the adipocyte differentiation. These expression profiles were similar to that of acetyl-CoA carboxylase-1, but not like to that of 3-hydroxy-3-methylglutaryl-CoA reductase. These results suggest that AACS in adipose tissue plays an important role in utilizing ketone body for the fatty acid-synthesis during adipose tissue development.


*Keywords:* Acetoacetyl-CoA synthetase; Ketone body; Tissue distribution; Sex difference; Adipose tissue; Adipogenesis; Cell differentiation; mRNA expression

In the mitochondria, ketone bodies, D(−)-β-hydroxybutyrate and acetoacetate are important energy source, and succinyl-CoA:3-ketoacid CoA transferase (SCOT; EC 2.8.3.5) has been regards as for the ketone body utilization in the mitochondria [1]. On the other hand, in the cytosol, ketone body is known to be activated and incorporated into cholesterol and fatty acids by acetoacetyl-CoA synthetase (AACS, acetoacetate-CoA ligase, EC 6.2.1.16), which we purified for the first time from bacterium, Zoogloea ramigera I-16-M [2], and rat liver cytosol [3]. This enzyme is the strictly acetoacetate-specific ligase [4,5]. Endemann et al. [6] have proposed that main function of AACS is direct supply of acetyl-CoA from acetoacetate in the cytosol for the synthesis of the lipidic substances and that such cytosolic activation could bypass the pathway involving ATP-dependent supply of acetyl-units from mitochondria to cytosol.

Previously, we showed that the AACS activity in the liver of rats remarkably increased and then decreased during the animal's development [5] and increased upon the administration of hypocholesterolemic compounds, cholestyramine and/or pravastatin, to rats [7]. We have also demonstrated that hepatic AACS activity markedly decreased in streptozotocin-induced diabetic rats as in the case of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) or acetyl-CoA carboxylase [8]. Furthermore, we found that AACS mRNA in the cerebellum was restricted to glial cells, while in the cerebral cortex, it was restricted to neural cells [9]. These results suggest that direct activation of acetoacetate by AACS in the cytosolic compartment of the cells is an important step for the regulation of ketone body utilization on cholesterol and/or fatty acids biosynthesis.

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In order to clarify a physiological role of AACS in the lipogenesis, we examined expression levels of AACS mRNA in a variety of rat lipogenic tissues. The present manuscript describes the remarkable expression of AACS mRNA in male subcutaneous white adipose tissue among the lipogenic tissues, its preferential expression in mature adipocytes, and the alteration of its level during the adipocyte-differentiation.

## Materials and methods

*Preparation of RNA.* RNA was prepared from rat tissues using a QuickPrep total RNA extraction kit (Amersham Biosciences), and from primary adipocytes using a RNeasy mini kit (Qiagen).

*Northern blotting analysis.* Aliquots of RNAs (10 μg each) were dissolved on a denaturing agarose gel (1%) containing formaldehyde and transferred to a nylon membrane in 20× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate) overnight. The membrane was prehybridized at 42 °C for 4 h in a hybridization solution (10% dextran sulfate, 50% formamide, 2% SDS, 5× SSPE, 5× Denhardt's solution, and 100 μg/ml heat-denatured salmon sperm DNA), followed by hybridization at 42 °C for 18 h in the hybridization solution containing a $^{32}$P-labeled probe labeled by Ready-To-Go DNA Labeling Beads (Amersham Biosciences) with cytidine 5′-[α-$^{32}$P]dCTP (~30 TBq/mmol, Amersham Biosciences). The membrane was then washed at room temperature three times for 5 min each time in 2× SSC/0.1% SDS, and at 65 °C twice for 30 min each time in 0.25× SSC/0.1% SDS. The washed membrane was analyzed with a radioimaging analyzer (BAS2000, Fuji Photo Film, Japan).

*PT-PCR for PPARγ.* Aliquot of RNAs (4 μg) was incubated for 60 min at 37 °C in the reaction mixture (20 μl) containing 300 U of moloney murine leukemia virus reverse transcriptase (Gibco-BRL, USA), 15 U of human placenta RNase inhibitor (WAKO), and 0.5 μg of random hexadeoxynucleotide primer (Takara). To amplify the human AACS cDNA fragment, PCR was performed for 30 cycles in the reaction mixture containing an aliquot of the above cDNA solution, 0.05 U/μl EX *Taq* DNA polymerase (Takara) and 4 pmol/μl each of sense (gagatgccattctggcccaccaacttcgg) and antisense (tatcataaataagcttcaatcggatggttc) primers of rat PPARγ (Accession No.: AF156666) [10].

*Preparation of probes.* The cDNA fragments of AACS, SCOT, HMGCR, and ACC1 were amplified from rat liver cDNA. The oligonucleotide primers used for amplification were: forward (atgtccaagctggcacggct) and reverse (ttcagaagtcctgcagctca) for rat AACS (GenBank Accession No. AB026291) [7]; forward (atgacnccncarggnacntt) and reverse (gtccgtgtctgaagacaact) for human SCOT (GenBank Accession No. P55809) [11]; forward (acggtgacacttactatctg) and reverse (accatgtgacttctgacaag) for rat HMGCR (GenBank Accession No. NM013134); forward (gtccgtgtctgaagacaact) and reverse (tcctaactgttccagagct) for rat ACC1 (GenBank Accession No. J03808) [12]. The cDNA fragment of leptin was amplified from rat epididymal adipose tissue. The oligonucleotide primers used for amplification were: forward (tgctccagcagctgcaaggt) and reverse (gaagaatgtcctgcagagag) for rat leptin (GenBank Accession No. NM013076) [13]. The fragments were cloned into the pGEM-T vector (Promega).

*Preparation of mature adipocytes and stromal–vascular cells.* Mature adipocytes and stromal–vascular cells were prepared from rat subcutaneous white adipose tissue essentially according to the method of Ogawa et al. [13].

*Primary culture of preadipocytes.* Primary preadipocytes were cultured and differentiated according to Mitchell et al. [14]. The stromal–vascular cells were suspended in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented penicillin (100 U/ml; Gibco-BRL) and streptomycin (100 μg/ml; Gibco-BRL), and re-filtered. The cells were adjusted to a density of $4.0 \times 10^5$ cells/ml in DMEM with 10% fetal bovine serum (FBS; Gibco-BRL). Two milliliters volumes of these cells was plated onto 35 mm dishes. After 1 day in culture at 37 °C in an atmosphere of 5% $CO_2$, differentiation was induced by the addition of medium supplemented with isobutylmethylxanthine (IBMX; 0.5 mM; Sigma), dexamethasone (DEX; 0.25 μM; Nacalai tesque), and insulin (10 μg/ml; Sigma). After 48 h, the induction medium was removed and replaced by DMEM containing 10% FBS supplemented with insulin (10 μg/ml) alone [15]. This medium was changed every 2 days.

## Results and discussion

### *Expression of AACS mRNA in rat lipogenic tissues*

Fig. 1 shows the expression levels of AACS mRNA in male and female rat lipogenic tissues: brain, liver, white adipose tissue, and brown adipose tissue. The white adipose tissue was prepared from the subcutaneous region, and the brown adipose tissue from the interscapular region. The integrity of RNAs was confirmed by electrophoresis on a denaturing agarose gel containing formaldehyde (lower lane). The labeled probe hybridized to an mRNA of about 4.7 kb in all tissues examined. AACS mRNA was particularly abundant in male white adipose tissue. But in other lipogenic tissues, such remarkable sex difference of AACS expression level was not observed. SCOT, that is known as a key mitochondrial enzyme in the energy production from ketone bodies in various tissues except liver [16], was equally expressed in all tissues examined except for the liver. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR; EC 1.1.1.34), the rate-limiting enzyme of

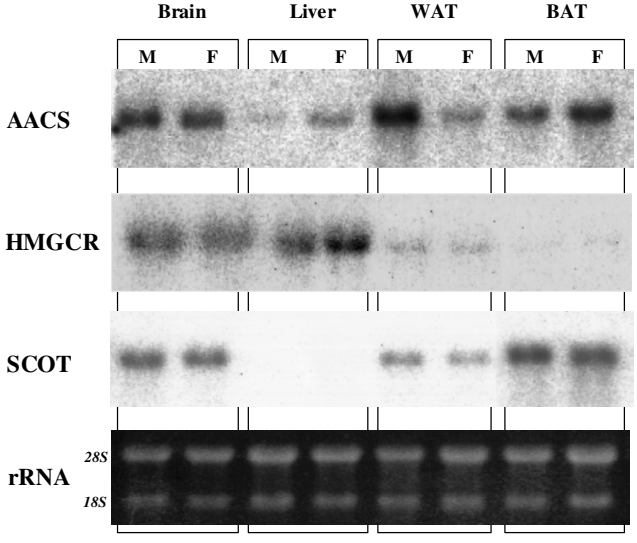


Fig. 1. Gene expression of acetoacetyl-CoA synthetase mRNA in rat lipogenic tissues. Total RNAs were isolated from brain, liver, white adipose tissue (WAT), and brown adipose tissue (BAT) of male and female rats (8-week old). Aliquots of RNAs (10 μg) were subjected to Northern blotting analysis of AACS, SCOT, and HMGCR cDNA probes. M and F indicate male and female, respectively. The lower panel shows EtBr staining of ribosomal RNAs (2 μg/lane).