

ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species

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Abstract Fatty acid synthase (FAS), a key lipogenic enzyme, is expressed in the two major sites of fatty acid production in the body, that is, the liver and the adipose tissue. Surprisingly, the relative contribution of these sites to lipogenesis is highly variable among species. For example, besides the situation in rodents, where liver and fat are equally active, lipogenesis in some mammals such as the pig occurs principally in adipose tissue, whereas in avian species, the liver is the main lipogenic site. We addressed the question concerning the factors determining the site of fatty acid synthesis. We show that the expression of adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein (ADD-1/SREBP-1) mRNA, but not SREBP-2, is linked to FAS protein content or activity in adipose tissues and livers of pig, chicken, and rabbit. Tissue differences in ADD-1/SREBP-1 mRNA expression between species were paralleled by commensurate variations in the nuclear concentration of SREBP-1 protein. Moreover, overexpression of ADD-1/SREBP-1 by adenoviral gene transfer induces FAS in chicken adipocytes, where lipogenesis is normally low. Conversely, the expression of a dominant negative form of ADD-1/SREBP-1 in pig adipocytes downregulates FAS expression. These results reinforce the role of ADD-1/SREBP-1 as a key regulator of lipogenesis, by extending its importance to nonrodent mammals and birds. Furthermore, they establish that differential expression of ADD-1/SREBP-1 is a key determinant of the site of fatty acid synthesis in the body.—Gondret, F., P. Ferré, and I. Dugail. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. *J. Lipid Res.* 2001. 42: 106–113.

Supplementary key words rabbit • pig • chicken • fatty acid synthase • gene regulation • liver • adipose tissue • adenovirus

Lipogenesis, that is, de novo synthesis of free fatty acids, is a key metabolic pathway for energy homeostasis in higher animals. Two major tissues produce fatty acids in the body: the liver and the adipose tissue. The fatty acids synthesized in the liver are exported through lipoprotein production, and thus provide an energy source and structural components for membrane building. In adipose tissue, de novo fatty acid synthesis directly contributes to in

situ fat deposition and long-term energy storage. Lipogenic flux is tightly controlled by hormonal and nutritional conditions. The importance of the nutritional status in the regulation of lipogenesis in liver and adipose tissue is underlined in many studies [for reviews see (1) and (2)]. Briefly, feeding a high carbohydrate diet induces, whereas fasting or fat feeding strongly inhibits, lipogenesis. A close relationship exists between the rate of fatty acid synthesis and the activity of the fatty acid synthase (FAS), a key lipogenic enzyme. Studies performed in various mammalian and avian species (3, 4) have led to the conclusion that FAS is controlled primarily at the level of gene transcription.

The general view that lipogenesis is similarly regulated in all tissues (liver and adipose tissue) comes from the situation in rodents. However, under permissive nutritional conditions, some tissue selectivity in the induction of lipogenesis can be observed in other species. For example, lipogenesis in birds is active exclusively in liver (5), whereas in pigs adipose tissue is by far the main lipogenic organ (6, 7) with little contribution from the liver. Such differences in the contribution of liver and adipose tissue to the whole body lipogenesis cannot be due to nutrient availability, and thus remains unexplained. This question is addressed in the present study.

Adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding proteins (ADD-1/SREBP), which belong to the helix-loop-helix-leucine zipper (bHLH-LZ) family of transcription factors, have been identified as potent activators of lipogenic gene expression. ADD-1/SREBP have been characterized as conditional *trans*-acting factors, activated by proteolytic cleavage

Abbreviations: ADD-1/SREBP, adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein; bHLH-LZ, basic helix-loop-helix leucine zipper; DMEM, Dulbecco's modified Eagle's medium; FAS, fatty acid synthase; PCR, polymerase chain reaction.

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of their membrane-bound precursors (8). Several lines of evidence argue for the implication of ADD-1/SREBP in the control of FAS gene expression. First, overexpression of ADD-1/SREBP in cultured cell lines (9, 10) resulted in the activation of the cotransfected FAS promoter, through a functional E-box sequence targeted by SREBP (11, 12). Second, changing the content of ADD-1/SREBP in primary cultured cells by adenovirus-mediated gene transfer of either constitutively active or dominant negative forms of ADD-1/SREBP led to drastic changes in endogenous FAS gene expression in primary hepatocytes (13, 14) or adipocytes (11, 15). Finally, the importance of these factors in the control of lipogenic genes was established in vivo by overexpressing various ADD-1/SREBP isoforms in transgenic mice (16, 17). To date, three SREBP isoforms have been characterized, each exhibiting a distinct pattern of expression. The ADD-1/SREBP-1c isoform is largely predominant in lipogenic tissues, whereas the alternatively spliced SREBP-1a is present at significant levels mainly in cell lines (18). The SREBP-2 isoform is expressed from a separate gene (19) and is induced when cellular cholesterol is depleted (20, 21). All three isoforms are able to activate lipogenesis in transfected cells and transgenic mice, although with different potencies (17, 22).

In the present study, we observed that the relative distribution of ADD-1/SREBP-1 mRNA, but not SREBP-2, between adipose tissue and liver closely parallels that of FAS expression in various species including rodents (rabbit), nonrodent mammals (pig), and birds (chicken). We also show using adenovirus-mediated gene transfer that ADD-1/SREBP-1 expression was necessary for FAS gene expression in those species. These results demonstrate that differential expression of ADD-1/SREBP-1 is the key factor controlling the relative contribution of liver and adipose tissue to lipogenesis.

MATERIALS AND METHODS

Animals

Animal studies were conducted in compliance with the French guidelines for animal care and slaughtering. Male pigs (60 kg in body weight, 16 weeks of age), rabbits (2.3 kg in body weight, 10 weeks of age), and chickens (4–5 weeks of age) were obtained from the Institut National de la Recherche Agronomique (Paris, France). All the animals had ad libitum access to their normal standard pelleted diet. The three diets contained 2,400–3,000 kcal of metabolizable energy per kg, 15–16% crude proteins, and 3–3.5% ether extract. Diet for the rabbits also provided 14.7% crude cellulose, needed for their specific digestive system. Pig and chicken diets were mainly based on cereals (wheat and barley), representing about 60% of the total formula. The rabbit diet mainly contained cereals (35%), and alfalfa and beet pulp (33%). All animals were killed in the fed state. Immediately after slaughter, livers and internal adipose tissues at leaf, perirenal, or abdominal sites in pig, rabbit, or chicken, respectively, were excised and used for further analysis. Within species, the adipose tissue site was chosen because of its predominant contribution to lipid metabolism (23–25).

Cloning of partial cDNAs encoding pig, rabbit, or chicken ADD-1/SREBP-1 and SREBP-2

In each species, partial cDNA fragments, encompassing the conserved bHLH-LZ domain of SREBP, were obtained by reverse transcription of total RNA primed by random primers (pdN6), followed by two rounds of nested polymerase chain reaction (PCR) carried out with gene-specific primers.

For ADD-1/SREBP-1 cloning, primers for the first PCR were chosen as described by Shimomura et al. (18) for cloning mouse SREBP-1. For the second PCR, nested primers derived from the conserved human (GenBank accession number U00968) and hamster (U09103) SREBP-1 sequences were chosen in the flanking region of the bHLH-LZ domain, as follows: 5' primer 5'-CAGCCCCACTTCATCAAGGC(A/G)GACTCG-3', corresponding to amino acids 241 to 249 of human SREBP-1a, and 3' primer 5'-TCGATGGCCTTGCGCAAGACAGC-3', corresponding to amino acids 360 to 367 of human SREBP-1a.

For SREBP-2 cloning, primers for the first PCR were chosen as described by Shimomura et al. (18) for cloning mouse SREBP-2. For the second PCR, nested primers derived from conserved human (U02031) and hamster (U12330) SREBP-2 sequences were chosen in the flanking region of the bHLH-LZ domain, as follows: 5' primer 5'-CAGATTCCCTTGTTTTGACCACACTG-3', corresponding to amino acids 250 to 258 of human SREBP-2, and 3' primer 5'-GCCAGCTTCAG(C/T)ACCATGTTTC-3', corresponding to amino acids 392 to 397 of human SREBP-2.

All PCR products were cloned into pCR2.1, using a TA-TOPO cloning kit (Invitrogen, La Jolla, CA), and plasmid DNA was prepared with plasmid Maxi kits (Qiagen, Valencia, CA). Sequencing reactions were performed by the dideoxy chain termination method (T7 sequencing kit; Amersham Pharmacia Biotech, Saclay, France)

Recombinant adenoviruses

Recombinant adenoviruses were described previously (11, 14). They allow overexpression of two forms of rat ADD-1/SREBP-1 under the control of the cytomegalovirus promoter. The first vector encoded the N-terminal 403 amino acids of the transcriptionally active nuclear form of the wild-type rat ADD-1/SREBP-1c (Ad ADD-1). The second vector was a dominant negative version of ADD-1/SREBP-1 (Ad ADD-1-DN), in which Tyr-320 was turned to alanine (15). This mutation allows unaltered dimerization with endogenous ADD-1/SREBP-1, but eliminates the binding to DNA. The Ad vector Ad Null, the expression cassette of which contains the major late promoter with no exogenous gene, was used as control. The Ad vectors were propagated in 293 cells, purified by cesium chloride density centrifugation, and stored at -80°C until use.

Adenovirus-mediated gene transfer in isolated adipocytes

Adipocytes were isolated by collagenase treatment (26) of internal adipose tissues of fed chicken and pigs. Isolated adipocytes were washed three times with Dulbecco's modified Eagle's medium (DMEM). Freshly isolated fat cells (approximately $2-5 \times 10^5$ cells) were transduced with adenoviral vectors in 200 μl of DMEM for 1 h at 37°C . Then, 1 ml of DMEM supplemented with 10% fetal calf serum, 25 mM glucose, and antibiotics was added. The adipocytes were maintained in the same medium for 48 to 96 h after viral infection. High glucose concentrations in the medium were used in order to prevent glucose starvation. The multiplicity of infection (number of infecting viral particles relative to the number of fat cells) was calculated a posteriori after determining the number of adipocytes as described previously (11).

Total RNA isolation and Northern blot hybridization

Total cellular RNA was isolated from livers and adipose tissues by the guanidium thiocyanate method (27). Twenty micrograms of total cellular RNA was denatured in formamide and formaldehyde, and subsequently separated in 1% formaldehyde-agarose gels. RNA was transferred to Hybond N+ membrane (Amersham Pharmacia Biotech). Membranes were hybridized as previously described (28) with labeled probes obtained by random priming in the presence of [α -³²P]deoxycytidine triphosphate (RTS labeling; Life Technologies, Rockville, MD). A chicken ADD-1/SREBP-1 riboprobe was generated with a T7 polymerase kit (Promega, Madison, WI). A human 18S-riboprobe was used to normalize for loading of RNA samples. Autoradiograms were scanned and quantified with an image processor program.

Western blotting

Cytosolic supernatants were obtained by centrifugation (100,000 g, 1 h, 4°C) after homogenization of tissues or isolated

adipose cells in sucrose buffer. Nuclear extracts were prepared from liver or isolated adipocytes as described by Boizard et al. (11). Protein content was determined as described by Bradford (29), using ovalbumin as standard. Twenty micrograms of cytosolic proteins or 80 μ g of nuclear extracts was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred onto Hybond C nitrocellulose membrane (Amersham Pharmacia Biotech). Membranes were blocked in phosphate-buffered saline-Tween 20 (0.1% w/v) containing 5% nonfat dry milk, and FAS protein was probed with a previously characterized anti-rat FAS polyclonal antibody (28). Nuclear SREBP-1 was detected with a monoclonal antibody (IgG2A4) obtained from the American Type Culture Collection (Manassas, VA). Detection of signal was performed with the ECL Western blot detection kit (Amersham Pharmacia Biotech) with anti-rabbit or anti-mouse horseradish peroxidase-conjugated IgG as second antibody. Autoradiograms were scanned and quantified with an image processor program.

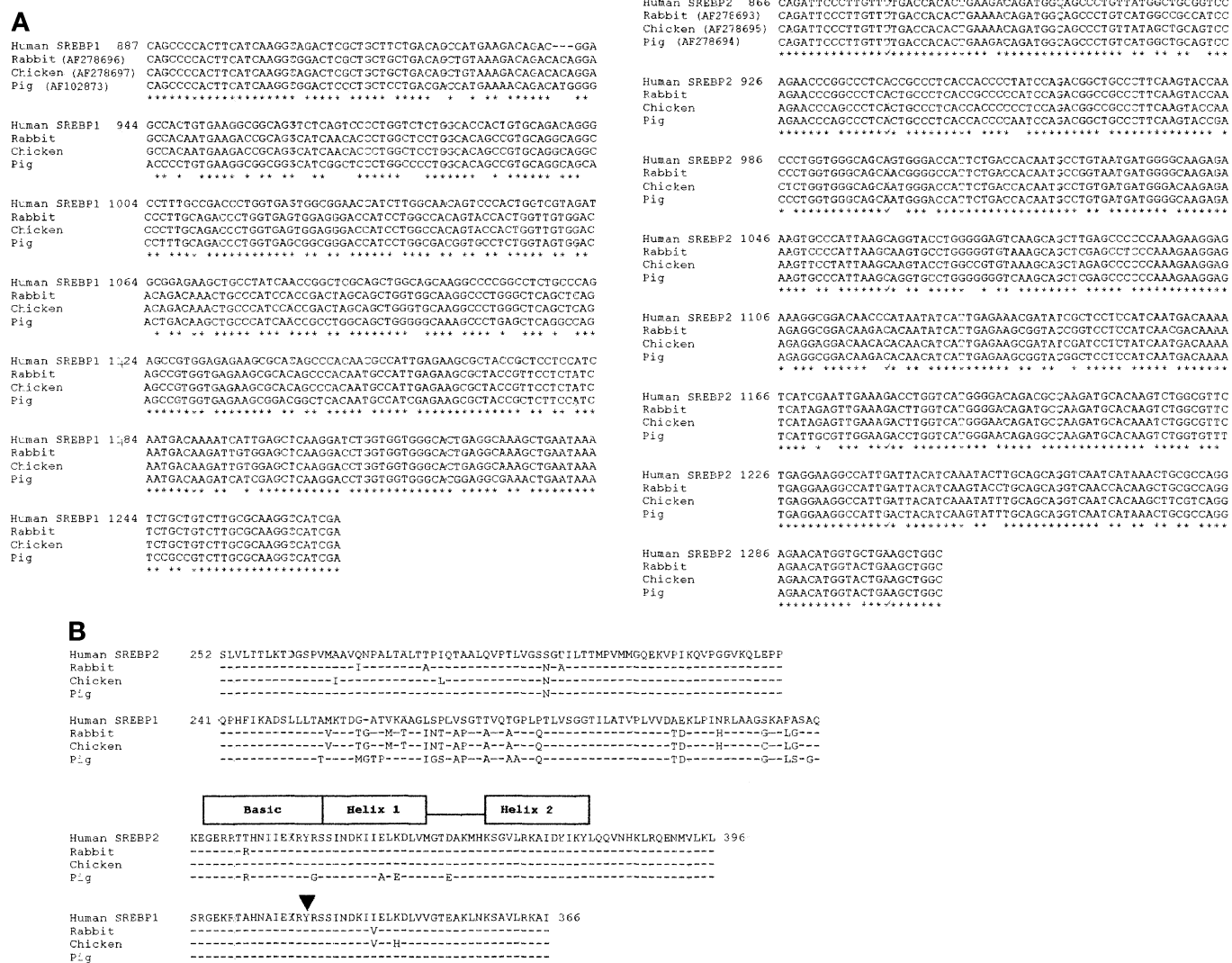


Fig. 1. Partial cDNA sequences of rabbit, chicken, and pig ADD-1/SREBP-1 and SREBP 2. A: An alignment of the rabbit, chicken, and pig sequences relative to human SREBP-1a or SREBP-2. Numbers indicate the nucleotide positions in the human sequences. B: The sequence of human SREBP-1 and SREBP-2 proteins and the amino acid changes observed in the translated sequences from rabbit, chicken, and pig. Numbers indicate the amino acid positions in the human sequences. The position of the bHLH domains are indicated as well as a critical tyrosine residue in the basic domain (arrowhead). These sequences have been deposited in GenBank, under the accession numbers AF278693–AF278697.

FAS activity determination

Fatty acid synthase activity was measured spectrometrically in the cytosolic supernatants, as previously described (28). Activities were expressed as nanomoles of nicotinamide dinucleotide phosphate, reduced form oxidized per minute per milligram of protein.

Statistical analysis

Data are presented as means + SEM. Comparisons within species were performed by Student's *t*-test analysis. Differences are considered significant at $P < 0.05$.

RESULTS

Level of ADD-1/SREBP-1 mRNA, but not SREBP-2 mRNA, correlates with the relative contribution of the liver and adipose tissue to FAS expression

We first amplify partial cDNA fragments encoding SREBP homologs in pig, chicken, and rabbit by reverse

transcriptase-PCR using RNA templates from the three species. The amplified products, encompassing the conserved bHLH domain of ADD-1/SREBP-1 and SREBP-2, were cloned and sequenced. **Figure 1** shows the alignment of these sequences relative to human SREBP-1 and SREBP-2. As expected, a high degree of homology to human SREBP-1 [84%, 84%, and 82% for chicken (GenBank AF278697), rabbit (GenBank AF278696), and pig, respectively] and to human SREBP-2 [91%, 90%, and 92% for rabbit (GenBank AF278693), chicken (GenBank AF278695), and pig (GenBank AF278694), respectively] was found. The pig ADD-1/SREBP-1 sequence is identical to that reported recently by Ding, McNeel, and Mersmann (30) (AF102873). At the amino acid level, sequence identity to human SREBP-1 and SREBP-2 was high (Fig. 1B), with a nearly perfect amino acid conservation in the helix-loop-helix motif. Notably, the tyrosine residue in the basic domain, which is critical for the dual

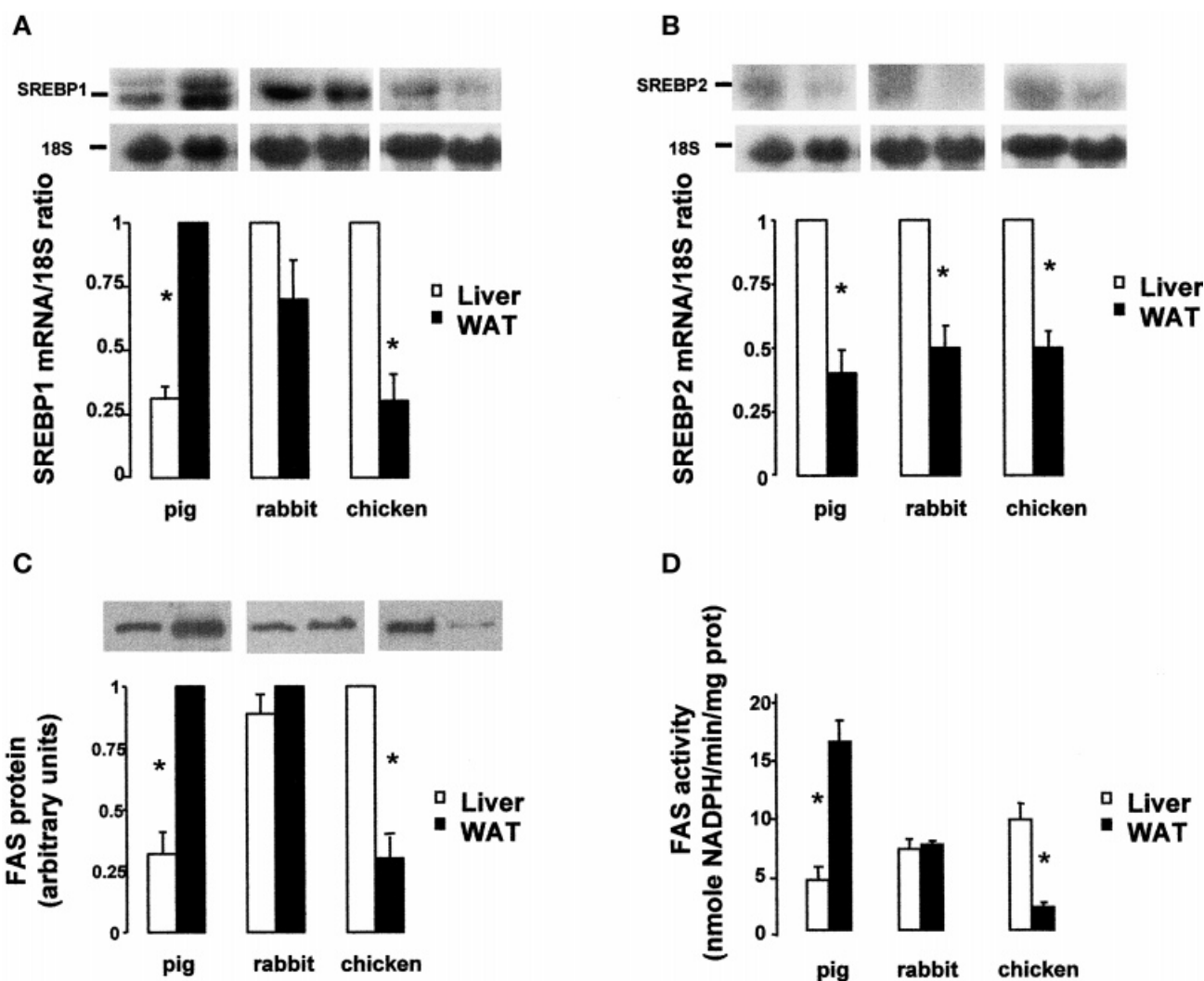


Fig. 2. Relative expression of ADD-1/SREBP-1, SREBP-2, and FAS in the liver and white adipose tissue (WAT) of pig, rabbit, and chicken. In (A)–(C), representative blots are shown together with the quantification of the signals obtained in three independent experiments. In (A) and (B), SREBP mRNA levels were normalized to 18S RNA. Within each species, the signals obtained in the tissue showing the higher expression were arbitrarily set to 1. No quantitative comparison can be made from one species to another. D: The activity of FAS assayed on cytosolic supernatants of liver and adipose tissue homogenates. An asterisk (*) indicates a significant difference in FAS activity between liver and adipose tissue ($P < 0.05$). All values are means + SEM obtained from at least three individuals.

binding specificity to E-boxes and SRE (15), was conserved in the three species.

These species-specific probes were used in Northern blots (Fig. 2) to assess the relative distribution of SREBP-1 and -2 between liver and adipose tissue. The hybridization pattern obtained for the pig ADD-1/SREBP-1 mRNA, that is, two bands of approximately 5 kb in length, is similar to that reported by us and others for the rat. In contrast, ADD-1/SREBP-1 message was detected as a unique band in rabbit and chicken. For SREBP-2 mRNA, the size and number of bands of the hybridization signals in all three species were similar to that observed by others in mice. The quantification of the signals obtained in liver relative to adipose tissue is shown in Fig. 2. ADD-1/SREBP-1 mRNA (Fig. 2A) is equally represented in liver and adipose tissue of the rabbit, whereas its expression is increased 3.5-fold in pig adipose tissue relative to liver. On the opposite, ADD-1/SREBP-1 expression in adipose tissue from chicken is 3-fold lower than that observed in liver. A similar analysis of the relative expression of SREBP-2 (Fig. 2B) shows that SREBP-2 mRNA levels were decreased 2-fold in adipose tissue versus liver in all three species. Interestingly, the species-specific distribution of liver and adipose tissue ADD-1/SREBP-1 mRNA closely parallels that of FAS. This is clearly shown in Fig. 2C and D, in which FAS expression was evaluated by Western blot analysis or by determination of its catalytic activity. Thus these data indicate that there is a strong correlation between the relative contribution of liver and adipose tissue to FAS activity and the expression of SREBP-1 but not SREBP-2 mRNA.

We next examined whether species differences in SREBP-1 mRNA distribution between liver and adipose tissue were followed by changes in the nuclear content of SREBP-1 protein. Figure 3 shows a 2-fold increase in the nuclear SREBP-1 content in adipose tissue versus liver in pig, and undetectable levels of SREBP-1 protein in adipose tissue from chicken. SREBP-1 protein amounts were slightly higher in liver than in adipose tissue in rabbit.

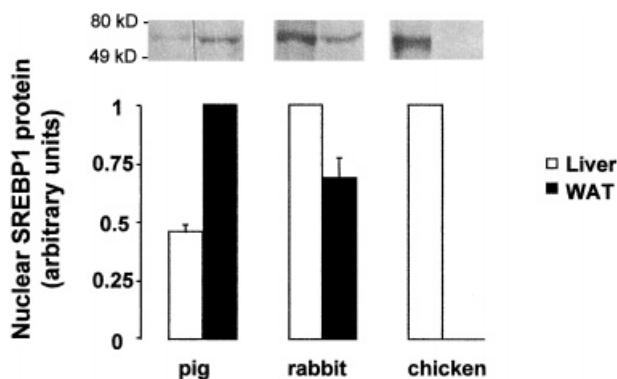


Fig. 3. Nuclear content of SREBP-1 protein in the liver and adipose tissue of pig, rabbit, and chicken. Eighty micrograms of protein was analyzed by Western blot as described in Materials and Methods. Representative Western blots are shown together with the quantification of the signals obtained in two independent experiments. Within each species, the signals obtained in the tissue showing the higher expression were arbitrarily set to 1.

ADD-1/SREBP-1 overexpression is sufficient to induce FAS expression in a tissue of low lipogenic capacity

To establish whether the parallelism between the relative expression of ADD-1/SREBP-1 in liver and adipose tissue, and that of FAS, might reflect an effective control of FAS expression by ADD-1/SREBP-1, we have manipulated the levels of ADD-1/SREBP-1 in cells differing in their lipogenic capacities by means of adenovirus-mediated gene transfer. Given the high degree of amino acid identity of the ADD-1/SREBP-1 protein in the different species, we hypothesized that the nuclear version of rat ADD-1/SREBP-1 should be transcriptionally active in the chicken, in which FAS promoter organization closely resembles that of rat (31). The active form of rat ADD-1/SREBP-1 was thus overexpressed in isolated chicken fat cells that normally exhibited low FAS activity. Figure 4 shows that FAS protein content measured on Western blots increased significantly on 2 days of infection of the adipocytes with the virus encoding ADD-1/SREBP-1, whereas no change occurred in the cells infected with the control null adenovirus. This indicates that raising the levels of transcriptionally active ADD-1/SREBP-1 is sufficient to induce the expression of FAS in a cell that normally expresses low levels of lipogenic enzymes

ADD-1/SREBP-1 expression is necessary to maintain high levels of FAS expression in active lipogenic cells

In a reciprocal experiment, we examined the effect of the suppression of endogenous ADD-1/SREBP-1 activity in cells that normally express high levels of ADD-1/SREBP-1 and FAS. Isolated pig fat cells were thus infected with a dominant negative form of rat ADD-1/SREBP-1 (ADD-1-DN), which has been previously characterized as a

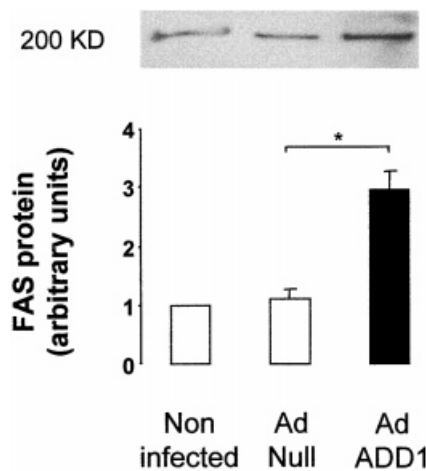


Fig. 4. Effect of the overexpression of ADD-1/SREBP-1 in chicken adipocytes. Freshly isolated adipocytes were infected by the adenovirus vector encoding (or not encoding) an active version of rat ADD-1/SREBP-1. One unit of multiplicity of infection represents 1,000 PFU/cell. FAS protein was quantified by Western blot after 2 days. A representative blot is shown, together with a quantitative representation of the results obtained in three independent experiments. Values are expressed as a ratio of the signal obtained in noninfected cells. Significant differences at the $P < 0.05$ level are shown (*).

potent suppressor of ADD-1/SREBP-1 transcriptional effects (15). The dominant negative activity of this form relies on a single amino acid change that eliminates binding activity, without altering homodimerization through the HLH domain. The ADD-1-DN form has been shown to inhibit dose dependently the binding of ADD-1/SREBP-1 dimer to its target DNA sequence by sequestering endogenous ADD-1/SREBP-1 as inactive complexes. Given the conservation of the critical tyrosine residue in the DNA-binding domain of pig ADD-1/SREBP-1, and the absence of any amino acid change between pig and rat ADD-1/SREBP-1 in the helix-loop-helix domain, which allows dimerization, we hypothesized that the rat ADD-1-DN should be efficient in suppressing pig ADD-1/SREBP-1 transcriptional activity. Isolated pig fat cells were thus infected with a virus encoding the rat dominant negative form of ADD-1/SREBP-1, and FAS activity was measured after 4 days. It must be emphasized that the level of FAS activity in those pig adipose cells was quite high and did not decline significantly, even after a 4-day period in a standard culture medium (data not shown). Results in Fig. 5A show that infection of the cells with increasing

titters of the control null adenovirus did not modify the level of FAS activity. In contrast, a dose-dependent decrease in FAS activity, reaching 40% at higher titers, was measured in cells infected with the ADD-1-DN virus. Figure 5B shows that the 40% decrease in FAS activity in cells infected with the higher titers of ADD-1-DN was accompanied by a proportional reduction in FAS protein content in Western blots. In these experiments, the inhibition of FAS gene expression was less profound than that observed previously in rat adipocytes, in which only 25% of the initial amount of FAS activity was recovered after a 4-day infection period. This can be due to the longer half-life of FAS protein in pigs than in rats. Alternatively, despite the large spectrum of species that adenovirus can infect, it is also possible that infection is less efficient in pig than in rat fat cells. Furthermore, a lower level of dominant negative expression in pig cells might also explain these differences. Nevertheless, the present data demonstrate that the expression of FAS in isolated pig adipocytes is strongly dependent on the presence of ADD-1/SREBP-1.

DISCUSSION

The importance of the ADD-1/SREBP family of transcription factors in the regulation of liver lipogenic genes has been established *in vivo*, in transgenic mice overexpressing various SREBP isoforms (16, 17, 22) and in ADD-1/SREBP-1 knockout mice, which are unable to exhibit normal lipogenic responses to nutritional manipulations (32). All these studies have addressed the question of the regulation of the lipogenic genes in rodent models, in which the liver and the adipose tissue equally contribute to the whole body lipogenesis and similarly respond to nutritional changes. However, some situations exist in which these two organs markedly differ in their lipogenic capacity, although they are subjected to an identical nutritional and hormonal environment. This is the case for nonrodent mammals and birds, in which only one lipogenic site is strongly active: the adipose tissue in pigs (4) and the liver in birds (3). The present study shows that in these two species, there is a close relationship between the tissue specificity of FAS expression and the level of ADD-1/SREBP-1 but not SREBP-2 mRNA. We also show that the tissue distribution of SREBP-1 mRNA between species is paralleled by commensurate variations in the nuclear concentration of SREBP-1 protein. This suggests a direct role for SREBP-1 in the relative level of FAS protein between tissues and species. However, some studies have reported the existence of posttranslational modifications such as phosphorylation (33), which might modulate SREBP transcriptional activity.

Our data on adenoviral gene transfer, showing that positive or negative changes in ADD-1/SREBP-1 protein induce parallel modulations of FAS gene expression, also strongly suggest a key role for SREBP-1 in the regulation of FAS expression in those species. In agreement, it should be noted that the site for SREBP binding is conserved on the FAS promoter of chicken (31). However, we

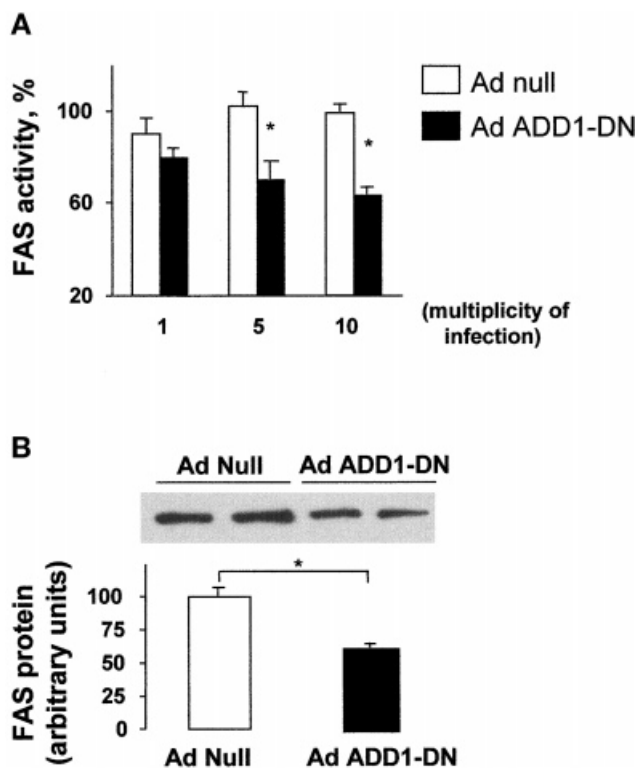



Fig. 5. Effect of the overexpression of a dominant negative form of ADD-1/SREBP-1 in freshly isolated pig adipocytes. Freshly isolated adipocytes were infected with the adenovirus vector encoding (or not encoding) a dominant negative version of rat ADD-1 (ADD-1-DN). FAS activity (A) and FAS protein (B) were determined after 4 days. In (A), FAS activity was expressed as a percentage of the activity measured in noninfected cells. Values are means \pm SEM obtained in three independent experiments. In (B), a representative immunoblot is shown, out of three independent experiments. The cells were infected at a multiplicity of infection of 10. Significant effects of ADD-1/DN are indicated ($*P < 0.05$).

cannot exclude that the positive effects of forced expression of SREBP-1 on the FAS gene might occur indirectly through substitution to an as yet unknown transcription factor. Alternatively overexpressed SREBP-1 could also modulate the expression of another *trans*-acting factor on the FAS promoter, or squelch nuclear factors involved in FAS gene transcription.

Taken as a whole, the present study reinforces the key role played by ADD-1/SREBP-1 in the control of lipogenesis, by extending, for the first time, its importance to non-rodent mammals and avian species. More importantly, our present work points out that tissue-specific expression of ADD-1/SREBP-1 is, at least partly, responsible for the partition of lipogenic gene expression between liver and adipose tissue. This raises the important question of the factors that regulate the tissue specificity of ADD-1/SREBP-1 expression and hence the site of fatty acid synthesis.

Our present knowledge of the regulation of the expression of ADD-1/SREBP-1 is far from complete. It has been shown that ADD-1/SREBP-1 expression in rat-cultured hepatocytes and adipocytes was increased by insulin (12, 14) and decreased by glucagon or cAMP (14). Furthermore, ADD-1/SREBP-1 abundance in the livers of mice was decreased by fasting (34), whereas insulin treatment of diabetic mice was able to induce ADD-1/SREBP-1 expression (35). These different experiments led to the conclusion that insulin is the main factor regulating ADD-1/SREBP-1 expression in rodents. This fits with the low lipogenic capacity of bird adipose tissue, which is poorly responsive to insulin (36, 37). On the other hand, insulin stimulates the activity of lipogenic enzymes in the liver of birds and in the adipose tissue of pigs *in vivo*, with a much lesser efficiency than in rats (38). This would suggest that insulin might not be the only factor regulating ADD-1/SREBP-1 expression. In agreement, some studies of the regulation of lipogenic gene expression in birds have pointed out the predominant role of thyroid hormones over that of insulin (39). The present study points out a differential regulation of ADD-1/SREBP-1 expression between liver and adipose tissue, suggesting that tissue-specific factors can cooperate with hormonal and nutritional regulators to control the expression of ADD-1/SREBP-1. 

We thank Fabienne Foufelle for the ADD-1 adenovirus, and Xavier Le Liepvre for assistance in sequencing. This work was supported by contract FAIR 97-3011 from the European Community.

Manuscript received 20 April 2000, in revised form 26 June 2000, and in revised form 11 September 2000.

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