Regulation of SREBP-1 expression and transcriptional action on HKII and FAS genes during fasting and refeeding in rat tissues

Yvan Gosmain, Nicolas Dif, Vanessa Berbe, Emmanuelle Loizon, Jennifer Rieusset, Hubert Vidal,[1] and Etienne Lefai

Unité Mixte de Recherche Institut National de la Santé et de la Recherche Médicale-U449/Institut National de la Recherche Agronomique-U1235, Institut Fédératif de Recherche (IFR) Laennec, Faculté de Médecine René Laennec, Université Claude Bernard Lyon-1, 69372 Lyon, Cedex 08, France

Abstract The sterol regulatory element binding protein 1 (SREBP-1) is regarded as a major factor involved in the nutritional regulation of lipogenesis. The aim of the present work was to demonstrate its involvement in the response of key genes of glucose and lipid metabolism in liver, adipose tissue, and skeletal muscle during fasting and refeeding. The regulation of hexokinase-2 (HKII) was investigated as a marker of the glucose metabolic pathway and that of FAS was investigated as a marker of the lipogenic pathway. The in vivo association of SREBP-1 with the promoter regions of these genes was determined in the different tissues using chromatin immunoprecipitation assays. Fasting decreased, and refeeding restored, FAS and HKII mRNA and protein levels in each tissue. The concomitant measurement of SREBP-1a and SREBP-1c mRNA levels, of mature SREBP-1 protein abundance in nuclear extracts, and of SREBP-1 interaction with target promoters led to the conclusion that SREBP-1 plays a major role in the response of FAS and HKII genes to nutritional regulation in rodents. These data elucidate the important role of SREBP-1 not only in the regulation of lipid metabolism but also of glucose metabolism and energy homeostasis.—Gosmain, Y., N. Dif, V. Berbe, E. Loizon, J. Rieusset, H. Vidal, and E. Lefai. **Regulation of SREBP-1 expression and transcriptional action on HKII and FAS genes during fasting and refeeding in rat tissues. *J. Lipid Res.* 2005.** 46: **697–705.**

Supplementary key words nutritional regulation • glucose metabolism • chromatin immunoprecipitation • gene expression • quantitative reverse transcriptase-polymerase chain reaction • lipogenesis • sterol regulatory element binding protein 1 • hexokinase-2 • fatty acid synthase

Sterol regulatory element binding proteins (SREBPs) are membrane-bound transcription factors of the basic-helix-loop-helix-leucine zipper family that have been shown to regulate gene expression of several enzymes implicated in cholesterol, lipid, and glucose metabolism (1, 2). Three members of the SREBP family have been identified and characterized (3). SREBP-1a and -1c are derived from a single gene through the use of alternative transcription start sites that produce different types of exon 1 (4).The third protein, SREBP-2, is derived from a separate gene (5). All SREBPs are synthesized as ~1,150 amino acid precursors bound to the endoplasmic reticulum and nuclear envelope (3). For its activation, the SREBPs undergo a sequential two-step cleavage process to release their NH_2-terminal segments that can then translocate to the nucleus (6). The mature form of SREBPs bind to sterol regulatory elements (SREs) in the promoter region of target genes to modulate their transcription (4, 7).

SREBP-1 has been implicated in the effect of insulin on the expression of key genes of lipid and glucose metabolism in different cell models, including hepatocytes, adipocytes, and muscle cells (8–12). Moreover, the expression of SREBP-1c was also found to be regulated by insulin (13–15), and SREBP-1 abundance is tightly related to the nutritional state in liver and adipose tissue (12, 16–18). These data suggested that SREBP-1, and mostly SREBP-1c, might directly be involved in the integration of nutritional changes and of insulinemia variations at the level of gene transcription (1, 2). This physiological role of SREBP-1 in response to nutrition was initially proposed for the control of lipogenic genes such as those coding for FAS and acetyl coenzyme-A carboxylase (ACC) in the liver and adipose tissue (1). Using in vivo chromatin immunoprecipitation (ChIP) assay, direct evidence for the involvement of SREBP-1 was recently provided through the demonstration of a diet-related modulation of its binding to the promoter region of FAS and ACC-2 genes in rodent liver (19–21). In addition to lipogenic tissues, SREBP-1 mRNAs and

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[1] To whom correspondence should be addressed.
e-mail: vidal@laennec.univ-lyon1.fr

proteins are expressed in skeletal muscle (14, 22), and recent work supports its role in the regulation of glucose metabolism-related genes, such as hexokinase-2 (HKII) (10, 11). Because SREBP-1 expression is also modulated by nutrition in muscle, with decreased levels in the fasted state and increased expression with feeding (23, 24), the central role of this transcription factor in the integration of nutritional changes might extend from lipogenesis to additional pathways, such as glucose metabolism in skeletal muscle.

The aim of the present work was to demonstrate the involvement of SREBP-1 in the response of key genes of glucose and lipid metabolism in liver, adipose tissue, and skeletal muscle during fasting and refeeding in rats. The regulation of HKII was investigated as a marker of the glucose metabolic pathway and that of FAS was investigated as a marker of the lipogenic pathway. The in vivo binding of SREBP-1 to the promoter regions of HKII and FAS genes was determined in the different tissues using ChIP assays. This is the first evaluation of the direct involvement of SREBP-1 on the expression of genes from distinct metabolic pathways, in different tissues, and in response to the fasting/refeeding transition in the same study. Our data strengthen the central role of this transcription factor in energy metabolism as a master regulator in response to nutritional changes.

MATERIALS AND METHODS

Animals and feeding protocol

Male Sprague-Dawley rats, weighing 225–250g, were obtained from Charles River (l'Arbresle, France). Rats were housed individually with a 14 h light/10 h dark cycle and adapted to the environment for 1 week before study. All procedures were performed in compliance with French legislation for the use of animals in public research laboratories and in accordance with the guide for the care and use of laboratory animals. After 1 week of consuming a starch diet (50% carbohydrate, 20% protein, and 5% lipids), one group (n = 5 animals) was maintained on standard diet (control group), one group (n = 5) was fasted for 48 h (fasted group), and one group (n = 5) was refed with a high-carbohydrate/low-fat diet (Research Diets, Inc., New Brunswick, NJ) for 20 h after 48 h of fasting (fasted/refed group). Animals were anesthetized with CO_2 and killed at the beginning of the light period. Blood samples were drawn for measurement of glucose, insulin, and nonesterified fatty acid concentrations, and muscle (flexor superficialis), epididymal adipose tissue, and liver were collected. Part of the tissue was frozen in liquid nitrogen for mRNA quantifications, and the remaining tissue was immediately used for nuclear protein preparations and ChIP assays.

RNA preparation and real-time RT-PCR

Total RNA from muscle and liver was isolated from ~100 mg of frozen tissue using TRIzol reagent (Invitrogen, Cergy-Pontoise, France), and total RNA from epididymal adipose tissue was extracted with the RNeasy minikit (Qiagen, Courtaboeuf, France), according to the manufacturer's instructions. The concentrations of HKII, FAS, SREBP-1a, SREBP-1c, and hypoxanthine phosphoribosyltransferase-1 (HPRT-1) mRNAs were determined by reverse transcription followed by real-time PCR using a Light-Cycler (Roche Diagnostics, Meylan, France). First-strand cDNAs were first synthesized from 1 μg of total RNA in the presence of 100 units of Superscript II (Invitrogen) using both random hexamers and oligo (dT) primers (Promega, Charbonnières, France). Real-time PCR was performed in a final volume of 20 μl containing 5 μl of a 60-fold dilution of the RT reaction medium, 15 μl of reaction buffer from the FastStart DNA Master SYBR Green kit (Roche Diagnostics), and 10.5 pmol of the specific forward and reverse primers (Eurobio, Les Ulis, France). Primers were selected to amplify small fragments (80–200 bp) and to hybridize in different exons of the target sequences. The list of the primers and real-time PCR conditions for each mRNA assay are available upon request (vidal@laennec.univ-lyon1.fr). For quantification, a standard curve was systematically generated with six different amounts (150–30,000 molecules/tube) of purified target cDNA cloned in the pGEM plasmid (Promega). Each assay was performed in duplicate, and validation of the real-time PCR runs was assessed by evaluation of the melting temperature of the products and by the slope and error obtained with the standard curve. The analyses were performed using Light-Cycler software (Roche Diagnostics) (25, 26). Results are presented as relative levels after normalization by HPRT-1 mRNA abundance.

Western blotting

SREBP-1 and Lamin B1 protein was determined by Western blotting in nuclear protein extracts. HKII, FAS, and α-tubulin proteins were analyzed in supernatants (10,000 *g* for 5 min) of whole tissue homogenates. Nuclear protein extracts were prepared from fresh tissue according to Azzout-Marniche et al. (13). For Western blotting, proteins were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and incubated with antibody directed against SREBP-1 (IgG-2A4; ATCC, Manassas, VA), HKII (C-14; Santa Cruz Biotechnology, Santa-Cruz, CA), or FAS (H-300; Santa Cruz Biotechnology). Detection was performed using the enhanced chemiluminescence system (Supersignal; Pierce, Rockford, IL). After analysis, the membranes were stripped (Re-Blot Plus; Chemicon International) and blotted with the α-tubulin antibody (TU-02; Santa Cruz Biotechnology) for total cell extract or with Lamin B1 (H-90; Santa Cruz Biotechnology) for nuclear protein extract to normalize for protein amount.

Formaldehyde cross-linking and ChIP

ChIP assays were performed according to the procedure described by Orlando, Strutt, and Paro (27), with minor modifications. Muscle, epididymal adipose tissue, and liver samples (~500 mg) were minced and treated for 10 min with formaldehyde (1% final concentration in phosphate-buffered saline) at room temperature. Cross-linking was stopped by the addition of glycine to a final concentration of 125 mM. Chromatin extracts was prepared from tissue homogenates and further fragmented by sonication. An aliquot was used to verify the fragmentation on agarose gels and to quantify the amount of DNA. Then, 50 μg of sonicated chromatin was first precleared for 1 h with protein A-Sepharose beads (Amersham Biosciences AB, Orsay, France). After centrifugation, supernatants were incubated overnight at 4°C with 8 μg of an anti-SREBP-1 antibody (H-160; Santa-Cruz Biotechnology) or 8 μg of anti-Sp1 antibody (PEP 2; Santa-Cruz Biotechnology) or no antibody (mock condition). The immunoprecipitated DNA/protein complex was bound to protein A-Sepharose beads during 3 h at 4°C and washed in a low-salt buffer, high-salt buffer, LiCl buffer, and Tris-EDTA buffer, in succession, as described by Duong et al. (28). Proteins were eliminated using proteinase K (200 μg; Promega) in the presence of 10% SDS by overnight incubation at 37°C. After phenol extraction, the DNA was precipitated, suspended in water, and used as a template for PCR. The sets of PCR primers used for the analysis of the rat HKII proximal promoter were 5′-CGTGAGCTGAGGTAGAGGTGGGCTC-3′

(HKII-S) and 5′-GCTGTTAATGAGCTTTGCCCAAGG-3′ (HKII-AS). Primers used for the analysis of the FAS proximal promoter were 5′-CCCAGTGTGACCAAGCACGCC-3′ (FAS-S) and 5′-GCGCTGGAGCACAAGGAACGC-3′ (FAS-AS). Real-time PCR analyses were performed using a Light-Cycler (Roche Diagnostics). PCR amplification products were also analyzed on ethidium bromide-stained 3% agarose gels. The specificity of the anti-SREBP-1 antibody (H-160) has been verified by Western blotting in nuclear extract proteins from rat liver. H160 antibody gave similar results as the IgG-2A4 antibody in samples from fed, fasted, and fasted-refed animals (data not shown). In addition, we previously reported that H160 antibody correctly recognizes SREBP-1 protein in muscle cells (10). Moreover, we verified that this antibody gave quantitative immunoprecipitation using different amounts of cross-linked chromatin (data not shown).

RESULTS

SREBP-1a and SREBP-1c expression is modulated by fasting and refeeding in rat tissues

Fasting for 48 h triggered significant decreases in plasma glucose and insulin concentrations and increases in nonesterified fatty acid and glycerol levels. Refeeding a high-carbohydrate diet after fasting was associated with marked increases in glycemia and insulinemia and with a reduction in the rate of lipolysis, as assessed by decreased concentrations of nonesterified fatty acid and glycerol (**Table 1**).

Figure 1 shows the diet-induced variations in SREBP-1a and -1c mRNA levels and in the protein abundance of SREBP-1 in nuclear extracts of liver, epididymal adipose tissue, and skeletal muscle. In the liver, SREBP-1a and SREBP-1c transcripts had a similar pattern of regulation, with reduction of their expression levels upon fasting and increase with refeeding to reach higher levels than in the control group (Fig. 1A). In liver, SREBP-1c was expressed ~10-fold higher than SREBP-1a, especially in the control and refed groups. The mature form of SREBP-1 protein, detected by Western blotting in extracts of nuclear proteins using a nonselective antibody, also showed a marked reduction during fasting and an overshoot upon refeeding. In the fasting group, the signal was too weak to be visualized with the exposition time used to reveal the blots. The two observed bands in liver nuclear extracts probably did not correspond to the 1a and 1c isoforms but rather to the previously reported cluster of proteins that characterizes mature SREBP-1 in this tissue (7).

In adipose tissue, SREBP-1a and SREBP-1c mRNA levels were significantly affected by fasting or refeeding. In contrast to the situation described in the liver, both mRNAs returned to control values upon refeeding (Fig. 1B). In this tissue, expression of SREBP-1c was ~3- to 5-fold higher than SREBP-1a in all nutritional situations. As in the liver, the abundance of SREBP-1 protein in nuclear extracts in adipose tissue was reduced upon fasting and strongly induced after refeeding.

In skeletal muscle, the two mRNAs encoding SREBP-1a and SREBP-1c were expressed at similar levels in the con-

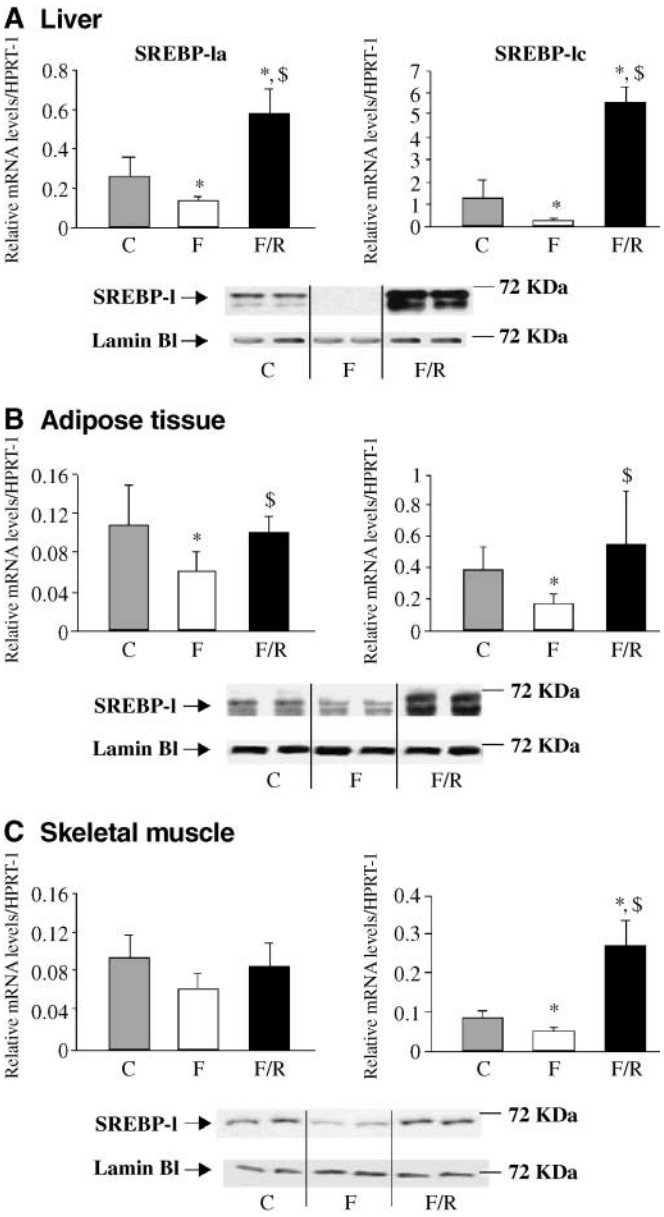

Fig. 1. Nutritional regulation of sterol regulatory element binding protein-1a (SREBP-1a) and SREBP-1c expression in rat tissues. The histograms represent SREBP-1a and SREBP-1c mRNA levels determined using RT-quantitative PCR and expressed as relative levels [hypoxanthine phosphoribosyltransferase-1 (HPRT-1) mRNA as a reference]. Data are means ± SEM (n = 5). For each tissue, a representative Western blot showing the abundance of SREBP-1 in nuclear protein extracts is shown below the mRNA data (Lamin B1 protein levels were determined as a control for loading). C, control group; F, fasting group; F/R, fasting/refed group. * $P < 0.05$ versus control animals; $ $P < 0.005$ versus fasted animals.

TABLE 1. Metabolic parameters of the three groups of rats

Variable	Control	Fasted	Fasted/Refed
Glucose (mM)	9.8 ± 1.0	7.1 ± 0.8[a]	10.5 ± 0.5[b]
Insulin (pM)	0.4 ± 0.06	0.2 ± 0.01[a]	2.5 ± 1.0[a,b]
Nonesterified fatty acid (μM)	311 ± 86	776 ± 59[a]	143 ± 17[a,b]
Glycerol (μM)	163 ± 9	252 ± 18[a]	123 ± 12[a,b]

Values are means ± SEM (n = 5).
[a] $P < 0.05$ versus the control group.
[b] $P < 0.05$ versus the fasted group.

trol group. Food deprivation or refeeding did not modify SREBP-1a mRNA levels in muscle, whereas SREBP-1c showed significant regulation with decreased levels upon fasting and an ~4-fold increase during refeeding (Fig. 1C). In the muscle nuclear extracts, the diet-induced changes in SREBP-1 protein were similar to those observed for SREBP-1c mRNA.

Regulation of FAS and HKII expression upon fasting/refeeding in rat tissues

FAS mRNA and protein levels were determined in liver, muscle, and adipose tissue and are represented in **Fig. 2**. In the control group, higher expression was found in adipose tissue and very low levels were found in skeletal muscle. Fasting for 48 h induced a significant decrease in FAS mRNA and protein in all tissues. Refeeding a high-carbohydrate diet triggered a dramatic increase of FAS expression in the liver both at the mRNA and protein levels (Fig. 2A). An increase in FAS mRNA was also observed in adipose tissue and, to a lesser extent, in skeletal muscle. Likely, the transition from fasting to refeeding was associated with a noticeable increase in FAS protein abundance in both adipose tissue and skeletal muscle (Fig. 2B, C).

We also investigated the changes in HKII expression as a marker of the glucose metabolic pathways. HKII was not detectable in liver, but it was highly expressed in adipose tissue and in skeletal muscle. As for FAS, fasting reduced HKII mRNA and protein levels and refeeding increased HKII expression compared with the fasting state in both tissues (Fig. 2B, C).

Fasting and refeeding modulated the binding of SREBP-1 to FAS and HKII gene promoters in rat tissues

We next examined whether the binding of SREBP-1 to the FAS and HKII promoters was affected in fasting/re-

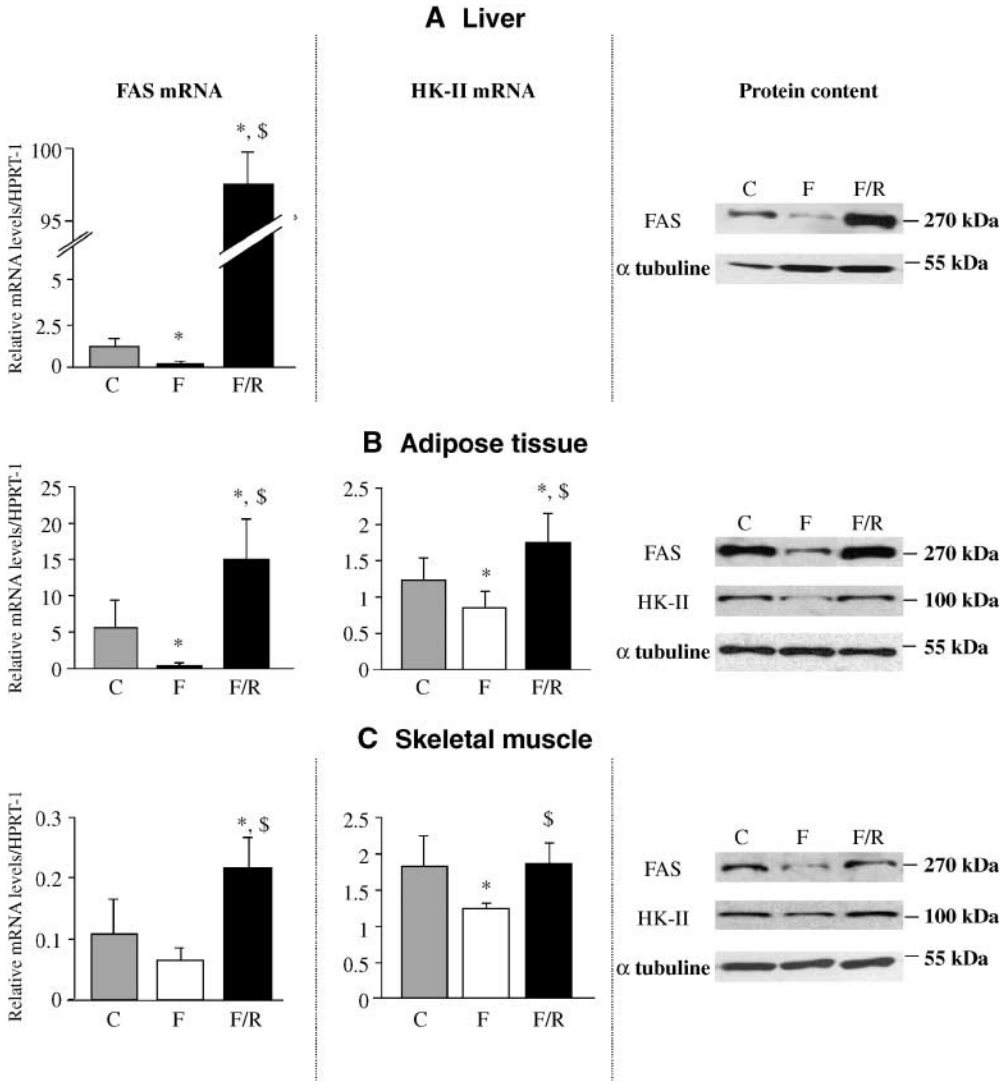

Fig. 2. Nutritional regulation of FAS and hexokinase-2 (HKII) expression in rat tissues. The histograms represent FAS and HKII mRNA levels determined using RT-quantitative PCR and expressed as relative levels (HPRT-1 mRNA as a reference). Data are means ± SEM (n = 5). For each tissue, a representative Western blot showing the abundance of FAS and HKII proteins in tissue homogenates is shown below the mRNA data (α-tubulin protein levels were determined as a control for loading). C, control group; F, fasting group; F/R, fasting/refed group. * $P < 0.05$ versus control animals; $ $P < 0.005$ versus fasted animals.

feeding conditions in the different tissues. Formaldehyde treatment of crude samples was used to cross-link the transcription factors bound to chromosomal DNA, and ChIP assays were performed using SREBP-1 antibody to immunoprecipitated fragments of DNA that were in interaction with this transcription factor when animals were killed. **Figure 3** shows the amplification by PCR of a specific portion of the proximal promoter of the FAS gene. The PCR products were analyzed on ethidium bromide-stained 3% agarose gels during the exponential phase of the PCR (33 cycles). The quantitative aspect of the PCR at 33 cycles was verified using serial dilution of the input, as shown in the lower part of Fig. 3. In addition, we also quantified the PCR products using real-time PCR and confirmed the observed differences between the nutritional conditions (data not shown). The binding of SREBP-1 to the FAS promoter was detectable in all tissues in the control group, although the binding was lower in the skeletal muscle than in adipose tissue or in the liver. Fasting reduced the binding of the FAS promoter in all tissues, with a drastic effect in liver, where the amplification product was no longer detected. When rats were refed with a high-carbohydrate diet, there was a marked increase in the association of SREBP-1 with the FAS promoter. In control experiments, immunoprecipitations using an anti-Sp1 antibody were performed in the same samples. PCR amplifications with the FAS primers are also shown in Fig. 3. There was no variation of Sp1 binding to the FAS promoter during the different nutritional situations. In each tissue, SREBP-1 binding to the FAS promoter matched the variations in mature SREBP-1 protein in nuclear extract (Fig. 1) and the variations in FAS mRNA levels (Fig. 2).

The interaction of SREBP-1 with the promoter of the HKII gene was also investigated. As for FAS, **Fig. 4** shows the PCR products analyzed during the exponential phase of the PCR (33 cycles) on ethidium bromide-stained 3% agarose gels. As for FAS, the quantitative aspect of the PCR at 33 cycles was verified using serial dilution of the input, as shown in the lower part of Fig. 4. We also quantified the PCR products using real-time PCR and confirmed the observed differences between the nutritional conditions (data not shown). Interestingly, there was no binding of SREBP-1 and Sp1 to the HKII promoter in the liver in any of the investigated conditions. Therefore, the binding of SREBP-1 to the HKII gene promoter was markedly affected by the dietary conditions in skeletal muscle and adipose tissue, in agreement with the regulated expression of HKII during fasting and refeeding (Fig. 2). As for

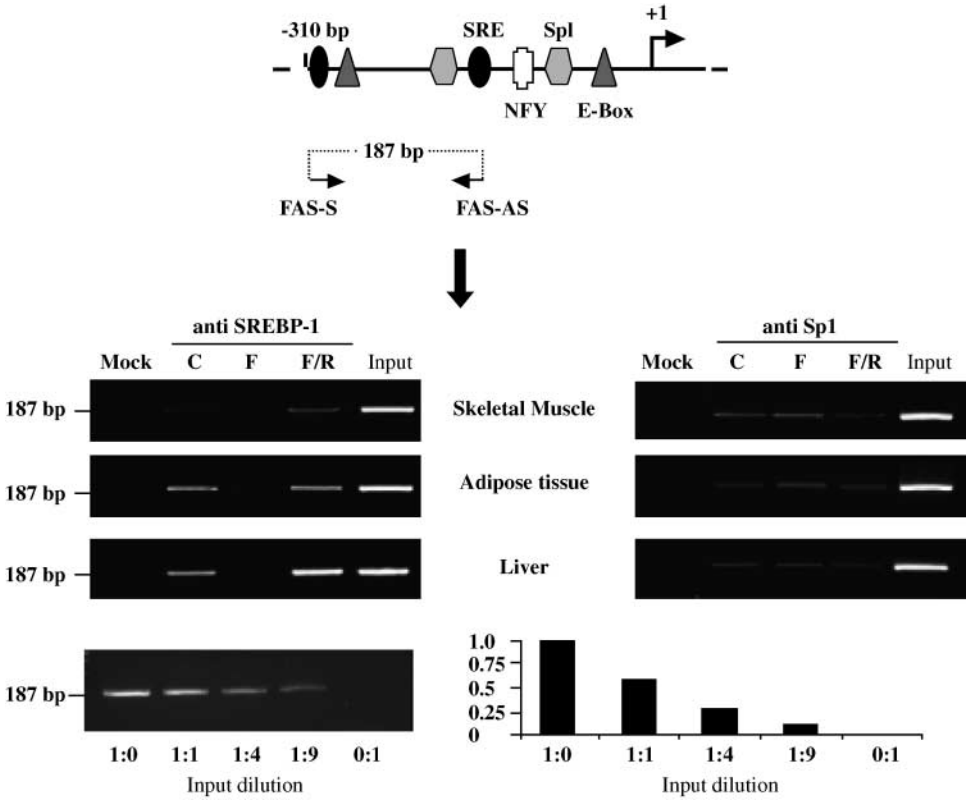

Fig. 3. Chromatin immunoprecipitation (ChIP) assay of SREBP-1 association with the FAS gene promoter. After cross-linking chromatin DNA to the interacting proteins, specific immunoprecipitation with anti-SREBP-1 and anti-Sp1 antibodies was performed as described in Materials and Methods. PCR products of the FAS promoter using the indicated primers were analyzed after PCR amplification (33 cycles). The PCR products were resolved on 3% agarose gels stained with ethidium bromide. C, control group; F, fasting group; F/R, fasting/refed group. The lower part of the figure shows verification of the quantitative aspect of the PCR amplification at 33 cycles using serial dilutions of the input.

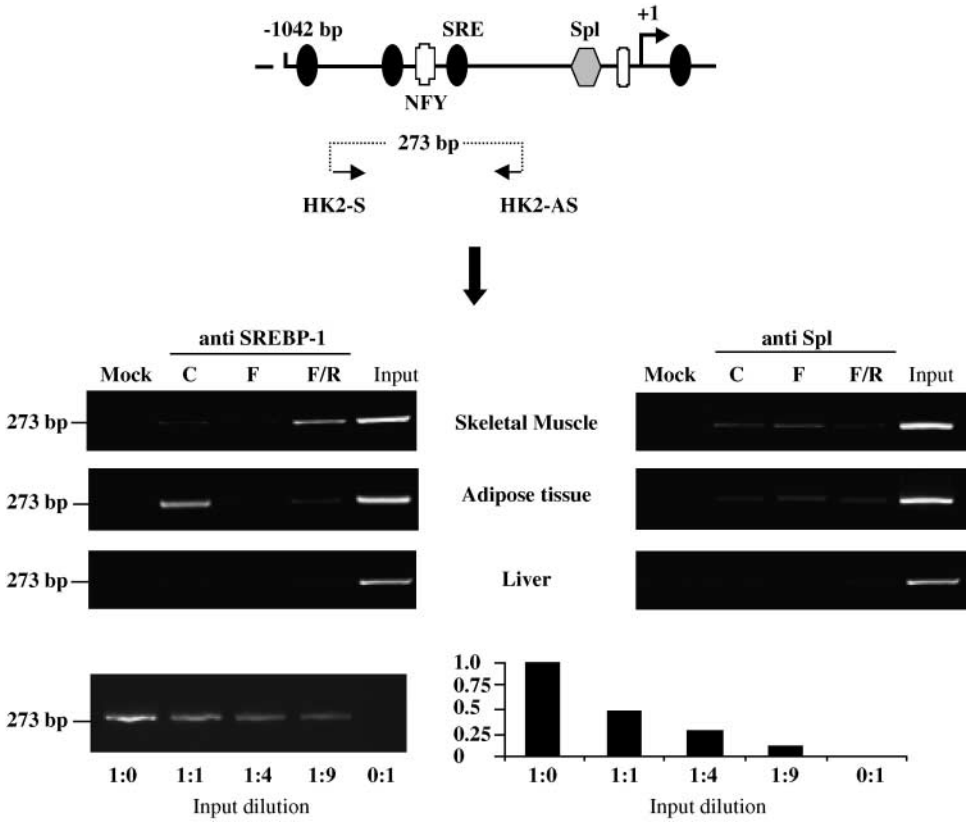

Fig. 4. ChIP assay of SREBP-1 association with the HK-II gene promoter. After cross-linking chromatin DNA to the interacting proteins, specific immunoprecipitation with anti-SREBP-1 and anti-Sp1 antibodies was performed as described in Materials and Methods. PCR products of the HK-II promoter using the indicated primers were analyzed after PCR amplification (33 cycles). The PCR products were resolved on 3% agarose gels stained with ethidium bromide. C, control group; F, fasting group; F/R, fasting/refed group. The lower part of the figure shows verification of the quantitative aspect of the PCR amplification at 33 cycles using serial dilutions of the input.

the FAS promoter, SREBP-1 binding was strongly reduced upon fasting, whereas refeeding a high-carbohydrate diet was associated with increased interaction in both tissues. In adipose tissue, the signal measured in the refed condition was lower than in the control group; however, there was a clear augmentation compared with the fasting situation. Therefore, as for FAS, the parallel changes in SREBP-1 binding to the HKII promoter and in HKII mRNA levels during fasting and refeeding strongly suggested that SREBP-1 is implicated in the regulation of HKII gene expression in skeletal muscle and adipose tissue.

DISCUSSION

A large body of data suggests that SREBP-1c is a crucial transcription factor involved in the regulation of the expression of key genes of lipid and glucose metabolism (1, 2). The works supporting this concept generally included experiments using overexpression of constitutively active or dominant negative forms of SREBP-1c (11, 29–31) or investigation of the promoter of target genes by transfection studies using reporter genes and in vitro DNA binding assays (30–33). However, such experiments do not necessarily reflect the in situ binding conditions of the transcription factor to the promoter sequences. Moreover, a number of studies have been performed using cell cultures (9, 11) that do not take into account the complex regulatory networks and the different pathways that can converge to control the expression of the target genes in vivo, such as during nutritional regulation. The ChIP assay allows the direct evaluation of transcription factor binding on gene promoters in vivo (27). Recently, this method has been applied to assess the interaction of SREBP-1 with FAS (21) and ACC-2 (34) gene promoters in rodent liver. These two studies clearly showed the binding of SREBP-1 to the promoters of these target genes. Moreover, they demonstrated that the binding of SREBP-1 is tightly regulated by nutrition, with strong reduction during food deprivation and increased association upon refeeding (20, 21). In the present study, we extend these previous findings and demonstrate that SREBP-1 association with target gene promoters also occurs in adipose tissue and skeletal muscle, two other important tissues for lipid and glucose metabolism. In addition, we provide the first evidence that SREBP-1 interacts in vivo with the promoter of HKII, a gene that is not directly related to lipogenesis. This clarifies the important role of SREBP-1 not only in the regulation of lipid

metabolism but also of glucose metabolism, as recently reviewed (1). We also demonstrate that the binding of SREBP-1 to the promoter regions of FAS and HKII genes is modulated by fasting and refeeding. This regulation occurred not only in the liver but also in adipose tissue and skeletal muscle, where SREBP-1 isoforms are expressed at lower levels. The changes in HKII expression upon fasting and refeeding are less important than the very robust changes in FAS expression. One possible explanation for this difference could be the promoter structures of these two target genes. The binding of SREBP-1 to its target sequence, and thus activation of gene expression, is higher when the target promoter possesses a SRE motif in close association with NFY and Sp1 elements (35, 36). This is the case for the FAS promoter (37) but apparently not for the HKII promoter (10). Alternatively, additional mechanisms could differentially affect HKII or FAS mRNA levels during nutritional changes, such as specific effects on mRNA stability or the involvement of additional transcription factors.

In agreement with previous data (12, 17, 22), we confirmed the presence of SREBP-1a and SREBP-1c mRNAs in the different rat tissues using RT-quantitative PCR assays. The transcripts encoding SREBP-1c were more abundant than those of SREBP-1a in the liver and in adipose tissue, the two lipogenic tissues. In skeletal muscle, both mRNAs were expressed at similar levels. In all tissues, SREBP-1c mRNA expression appeared to be more sensitive to the nutritional states than SREBP-1a. Nevertheless, significant changes in SREBP-1a mRNA levels during fasting and refeeding were also observed in the liver and adipose tissue, although of smaller magnitude than for SREBP-1c. Similar results were reported by Horton et al. (17) using an RNase protection assay in mouse liver. Interestingly, when measuring the amount of mature SREBP-1 protein in nuclear extracts, it clearly appeared that changes in nuclear SREBP-1 abundance follow the variations in SREBP-1c mRNA in the different tissues. This strongly suggested that SREBP-1c was the main isoform of SREBP-1 in the nuclear extracts. However, we could not exclude the possibility that low amounts of SREBP-1a were also present.

FAS is the key enzyme of de novo lipogenesis, catalyzing the conversion of acetyl-CoA and malonyl-CoA to palmitate. Nutritional regulation of FAS mRNA expression in rodent adipose tissue and liver has been previously documented (38, 39). We confirmed these data and also reported the variations of FAS protein during fasting and refeeding. FAS mRNA and protein were also detected in skeletal muscle, although at lower levels than in bona fide lipogenic tissues. Interestingly, fasting and refeeding affected the expression of FAS in rat skeletal muscle, suggesting possible regulation of lipogenesis by nutrition in rodent muscle. Regarding the implication of SREBP-1 in the transcriptional regulation of the FAS gene, the promoter region contains several putative binding sites for SREBP-1. It has been demonstrated that a SRE located at position −150 from the transcription start site binds SREBP-1 in vivo and in vitro and is probably involved in the response of the FAS gene to refeeding and insulin action (12, 21).

HKII catalyzes the phosphorylation of glucose into glucose-6-phosphate in insulin-sensitive tissue such as muscle, heart, and adipose tissue. The regulation of the expression of HKII by nutrition has not been extensively documented. Insulin has been shown to increase the expression of HKII in rodent and human muscle and adipose tissue (14, 40, 41), suggesting possible regulation during dietary conditions associated with changes in insulinemia. We found a reduced expression of both the mRNA and the protein levels of HKII upon 48 h of fasting. Refeeding with a high-carbohydrate diet after fasting restored HKII expression in muscle and in adipose tissue. We recently demonstrated that SREBP-1 induces HKII gene transcription in primary culture of human muscle cells (10). We confirmed here that SREBP-1 binds to the HKII gene promoter in vivo in adipose tissue and skeletal muscle. This interaction was decreased upon fasting and increased after refeeding. These data strongly suggested that SREBP-1 is involved in the nutritional regulation of HKII gene expression. The binding sites of SREBP-1 on the rat HKII gene promoter remains to be defined. We have identified a SRE site that binds SREBP-1 in vitro in the promoter region of the human HKII gene located at position −339/−330. Its mutation totally abolished insulin effect on HKII gene transcription (10). A region with similar structure and organization is located between −800 and −600 in the promoter of rat HKII genes. Its involvement in the interaction with SREBP-1 remains to be studied. It has been clearly demonstrated that HKII is the isoform of the hexokinases specifically expressed in muscles, adipose tissue, and heart and that this enzyme is absent in the liver (42). Interestingly, we were not able to detect any association of SREBP-1 and of Sp1 with the HKII promoter in liver chromatin. This suggested that the HKII promoter is not accessible to the transcription factors in hepatocytes, probably reflecting a chromatin structure associated with the lack of expression of this gene in liver cells.

Our data, together with the abundant literature on the role of SREBP-1 accumulated over the last 10 years, demonstrate that this transcription factor plays a central role in the control of key metabolic gene expression in response to nutrition (1, 2). This role was further described in SREBP-1 gene knockout mice, which were not able to respond correctly to the induction of a number of metabolic genes upon refeeding (43). In the present study, the concomitant determination, in each tissue, of SREBP-1a and SREBP-1c mRNA levels, of mature SREBP-1 protein abundance in nuclear extracts, and of SREBP-1 binding to target gene promoters strongly supported a major role of SREBP-1c rather than SREBP-1a in the response of FAS and HKII genes to nutritional regulation in rodents. Nevertheless, in a recent report, Bennett, Toth, and Osborne (44) demonstrated that SREBP-1a rather than SREBP-1c was the main regulator of FAS expression in CHO mutant cell lines. In addition, they also found that SREBP-2 could also be involved in the regulation of FAS expression (44). In the present study, we found that SREBP-1a mRNA was generally less abundant than SREBP-1c but that fasting and refeeding significantly affected its abundance in liver and adipose tissue. The changes in SREBP-1a mRNA were

globally similar to those observed for SREBP-1c mRNA. Unfortunately, because of the lack of specific antibodies, it was not possible to discriminate between the two isoforms in the nuclear extracts and in the ChIP experiments. Therefore, we cannot exclude a role of SREBP-1a in the regulation of gene expression during nutritional changes. In that context, it is important to keep in mind that SREBP-1a is a more potent transcriptional activator than SREBP-1c (45) and that the SREBP-1c promoter displays functional SRE motifs and thus can be considered a target gene of SREBP-1 (46, 47).

In summary, our work demonstrates the regulation by fasting and refeeding of the binding of SREBP-1 with FAS and HKII gene promoters in liver, adipose tissue, and skeletal muscle. SREBP-1 protein abundance in nuclear extracts and the binding of SREBP-1 to target promoters led to the conclusion that SREBP-1 plays a major role in the response of FAS and HKII genes to nutritional regulation in rodents. These data demonstrate the important role of SREBP-1 not only in the regulation of lipid metabolism but also of glucose metabolism and energy homeostasis. Nevertheless, this does not exclude the contribution of other transcription factors activated by additional pathways, such as carbohydrate-responsive element binding protein, which can be activated by glucose metabolites (48, 49), or liver X receptors and the peroxisome proliferator-activated receptors, which can respond to lipid metabolites (50).

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