Transcriptional regulation of muscle fatty acid-binding protein

Julie O. CAREY,*§ P. Darrell NEUFER,* Roger P. FARRAR,† Jacques H. VEERKAMP‡ and G. Lynis DOHM*

*Department of Biochemistry, East Carolina University School of Medicine, Greenville, NC 27858, U.S.A., †Department of Kinesiology, University of Texas, Austin, TX 78712, U.S.A., and ‡Department of Biochemistry, University of Nijmegen, NL-6500 HB Nijmegen, The Netherlands

Heart fatty acid-binding protein (H-FABP) is present in a wide variety of tissues but is found in the highest concentration in cardiac and red skeletal muscle. It has been proposed that the expression of H-FABP correlates directly with the fatty acid-oxidative capacity of the tissue. In the present study, the expression of H-FABP was measured in red and white skeletal muscle under two conditions in which fatty acid utilization is known to be increased: streptozotocin-induced diabetes and fasting. Protein concentration, mRNA concentration and transcription rate were measured under both conditions. The level of both protein and mRNA increased approximately 2-fold under each condition. The transcription rate was higher in red skeletal muscle than in white muscle, was increased 2-fold during fasting, but was unchanged by streptozotocin-induced diabetes. In addition to supporting the hypothesis that H-FABP is induced during conditions of increased fatty acid utilization, these findings demonstrate that the regulation of H-FABP expression may or may not be at the level of transcription depending on the stimulus.

INTRODUCTION

Fatty acid-binding proteins (FABPs) are a ubiquitously expressed family of cytosolic proteins with a molecular mass ranging from 14 to 15 kDa. Although individual family members differ in structure, tissue distribution and ligand specificity, they all share the property of binding low-molecular-mass hydrophobic and amphipathic ligands. At least ten unique family members have been described, including gastrotropin, mammary-derived growth inhibitor, five FABPs and three retinoid-binding proteins (Ockner et al., 1992). FABPs bind fatty acids, their CoA and carnitine esters and are thought to transport these ligands from the plasma membrane to the interior of the cell for mitochondrial and/or extramitochondrial oxidation (Veerkamp et al., 1991; Ockner et al., 1992). Thus it has been suggested that FABPs may be important in regulating fat oxidation.

Through peptide sequence analysis, the FABP found in skeletal muscle was shown to be identical with heart FABP (H-FABP) (Peeters et al., 1991). H-FABP has a wider tissue distribution than the other FABPs, but is found in the highest concentration in heart and red skeletal muscle (Heuckeroth et al., 1987; Watanabe et al., 1991). Assessment of H-FABP expression in various skeletal muscles suggests that the levels of both protein (Crisman et al., 1987; Claffey et al., 1987; Miller et al., 1988; Paulussen et al., 1989, 1990; Peeters et al., 1989; Veerkamp and van Moerkerk, 1993) and mRNA (Heuckeroth et al., 1987; Claffey et al., 1987) correspond to the red oxidative-fibre type content in a given muscle. Thus it appears that the tissue distribution of H-FABP seems to correspond with the degree to which a tissue utilizes fatty acids as a fuel source (Glatz et al., 1988; Veerkamp and van Moerkerk, 1993).

The cellular FABP level can be manipulated by various physiological conditions and pharmacological treatments. For example, liver FABP (L-FABP) increases 3-fold on high-fat diet, starvation and administration of hypolipidaemic drugs such as clofibrate (Bass, 1988; Veerkamp et al., 1991). In addition, the myocardial H-FABP content can be modulated by the type of dietary fat (Glatz et al, 1991), and both the myocardial and skeletal H-FABP levels can be increased by exercise training or testosterone treatment (Van Breda et al., 1992). In general, metabolic situations that increase fatty acid oxidation result in an increase in the level of FABP.

The aim of the present study was to investigate the effects of streptozotocin (STZ)-induced diabetes and fasting on the level of H-FABP in red and white skeletal muscle. Both of these conditions have been shown previously to increase fatty acid oxidation (Masoro, 1967; Schein et al., 1971), therefore we hypothesized that these conditions would also increase the level of H-FABP. In addition, we studied the mechanism of H-FABP expression under these two metabolic conditions by determining the steady-state mRNA level and rate of transcription originating from the H-FABP gene.

MATERIALS AND METHODS

Materials

All radiolabelled compounds were purchased from Du Pont–New England Nuclear. Hybond-N was obtained from Amersham Corporation. RQ1 DNAase, RNAasin and all restriction enzymes were purchased from Promega, Madison, WI, U.S.A. All other chemicals unless otherwise specified were of molecular-biology grade and purchased from Sigma or Fisher Scientific.

Animals and experimental design

Male Sprague–Dawley rats (300–350 g; CD strain, Charles River Laboratories, Wilmington, MA, U.S.A.) were either made diabetic by a single intraperitoneal injection of 75 mg/kg STZ, fasted for 3 days or maintained as controls. The STZ-induced diabetic animals were killed 7 days after injection. Blood glucose at the time of death averaged 543 ± 13, 158 ± 3 and 119 ± 3 mg/dl

Abbreviations used: FABP(s), fatty acid-binding protein(s); H-FABP, heart fatty acid-binding protein; STZ, streptozotocin; β-GPA, β-guanidinopropionic acid.

§ To whom correspondence should be addressed.

for STZ-induced diabetic, fasted and control animals respectively.

Antibody preparation

A peptide corresponding to the last 15 residues of the C-terminus of rat H-FABP was synthesized and conjugated to keyhole limpet haemocyanin. Then 500 μg of the peptide–keyhole limpet haemocyanin conjugate was dissolved in 500 μl of sterile 0.9% NaCl and then emulsified with 500 μl of Freund's complete adjuvant. This was injected intramuscularly into three adult New Zealand White rabbits. Three boosters of 500 μg of conjugate with incomplete adjuvant were given at 3-week intervals. After 3 weeks, a final 250 μg booster was given with incomplete adjuvant. At 2 weeks after the final booster, blood was obtained by intracardiac puncture, and incubated at 37 °C for 1 h. The blood clot was loosened from the tube with a wooden stick and incubated at 4 °C overnight. The blood clot was removed from the tube carefully, after centrifugation at 5000 *g* (5000 rev./min) for 10 min in an IEC Centa-7 centrifuge (International Equipment Company); the resultant serum was utilized for Western blotting without further purification.

Protein preparation and Western-blot analysis

After the rats had been killed, quadriceps from one hindlimb were rapidly excised, red and white portions separated, frozen with tongs cooled in liquid nitrogen, and stored at −70 °C until analysis. After the muscle had been powdered using a cold steel mortar and pestle, 100 mg of muscle was homogenized in 1 ml of 20 mM Hepes buffer containing 1 mM EDTA and 250 mM sucrose using a Polytron (Brinkmann Instruments). The homogenate was centrifuged at 100000 *g* for 1 h at 4 °C in a Beckman 50Ti rotor. The protein concentration of the supernatant, containing the cytosolic fraction, was determined using the bicinchoninic acid assay from Pierce. Tricine/SDS/PAGE was performed as described by Schagger and von Jagow (1987), in order to maximize the resolution of low-molecular-mass proteins. The system of Hjerten (1962), used to denote the composition of the gels, defines T as the total percentage concentration of both monomers (acrylamide and bisacrylamide) and C as the percentage concentration of the cross-linker relative to the total concentration T. The 16.5% T/6% C separating gel and the 4% T/3% C stacking gel both consisted of 1.0 M Tris, pH 8.45, and 0.1% SDS. A separate anode buffer (0.2 M Tris, pH 8.9) and cathode buffer (0.1 M Tris, 0.1 M tricine, 0.1% SDS, pH 8.25) were utilized. Electrophoresis was performed at 10 °C, starting at 30 V, and increasing to 105 V after the samples entered the stacking gel, and continued for 16 h. Gels were electrically transferred to Immobilon (Millipore) membrane at 0.25 A for 75 min. Gels were stained with Coomassie Blue to check the completeness of transfer. Blots were blocked in 5% (w/v) dried milk and incubated with FABP antibody for 1 h at a 1:5000 dilution. Blots were washed in TBS (10 mM Tris/HCl, 0.9% NaCl, pH 7.4) and TBS/Tween [10 mM Tris/HCl, 0.9% NaCl, 0.05% (v/v) Tween 20, pH 7.4] repeatedly, and then incubated in donkey anti-rabbit–horseradish peroxidase conjugate antibody for 1 h. Blots were washed in TBS and TBS/Tween again and developed using the ECL (enhanced chemiluminescent) detection system (Amersham). Adjustment of exposure time generated films in the predetermined linear range. Resultant films were quantified by densitometric scanning performed on a computer-controlled laser densitometer (Ultrascan XL, LKB Pharmacia). Absorbance units from densitometric scanning of multiple films were normalized by inclusion of a rat heart standard on each gel.

RNA isolation and Northern-blot analysis

Total RNA was isolated from 200 mg of the powdered red or white quadriceps by the guanidinium thiocyanate/phenol/chloroform extraction method (Chomczynski and Sacchi, 1987) as modified by Puissant and Houdebine (1990). RNA (10 μg) was denatured and size-fractionated on a 1.25% agarose/2.0 M formaldehyde gel. After staining (0.5 μg/ml ethidium bromide), the 28S and 18S ribosomal bands were visualized and photographed by u.v. transillumination to ensure that the RNA was intact and evenly loaded. RNA was then transferred to Hybond-N, u.v. cross-linked, and prehybridized at 47 °C for 4 h in a solution of 50% (v/v) deionized formamide, 4 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate), 5 × Denhardt's solution (50 × Denhardt's = 0.1% each of BSA, poly(vinyl pyrrolidone) and Ficoll], 0.1 mg/ml yeast tRNA, 50 mM sodium phosphate (pH 7.0), 0.5 mg/ml sodium pyrophosphate and 1% SDS. Hybridizations were carried out overnight in hybridization solution (prehybridization solution with 1 × Denhardt's solution) at 47 °C using the appropriate cDNA probe at 1×10^7 c.p.m./ml. All cDNA probes were labelled with [α-^{32}P]dATP (3000 Ci/mmol) by random priming as previously described (Feinberg and Vogelstein, 1983). Blots were washed for 30 min in 0.1 × SSC/0.1% SDS at room temperature followed by 30 min at 55 °C, and then subjected to autoradiography using Kodak XAR-5 film with intensifying screens. The resulting autoradiograms were quantified by densitometry.

Isolation of nuclei

Nuclei were isolated from rat skeletal muscle by a modified procedure described by Zahradka et al. (1989). Quadriceps and gastrocnemius/plantaris muscles were dissected, pooled, minced and weighed (8–9 g). All procedures were carried out at 4 °C. The tissue were then immediately homogenized with a Polytron homogenizer in 31 vol. of lysis buffer (10 mM Hepes, pH 7.5, 5 mM KCl, 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol) containing 0.32 M sucrose. The homogenate was passed through four layers of cheesecloth and filtered through a 100-mesh stainless-steel screen (Fisher Scientific). Nuclei were collected by centrifugation at 1000 *g* for 10 min in a Sorval GSA rotor. The nuclear pellet was resuspended by gently triturating with 35 ml of lysis buffer containing 2.2 M sucrose and centrifuged for 90 min at 80000 *g* (27000 rev./min), in a Beckman SW28 rotor. The resulting nuclear pellet was gently rinsed with lysis buffer and resuspended in 2 ml of storage buffer (75 mM Hepes, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.5 mM dithiothreitol, 0.1 mM EDTA, 0.1 mM EGTA and 40% glycerol). The nuclei were repelleted by centrifugation for 10 min at 5000 *g*, in an IEC B-20A floor centrifuge, and were resuspended by thoroughly triturating in 200 μl of cold storage buffer, transferred to Microfuge tubes and stored at −80 °C until analysis.

Run-on transcription analysis

The procedure used for nuclear run-on analysis is based on techniques previously described by Cornelius et al. (1990). After thawing on ice, the concentration of DNA in each preparation was determined by lysing a 10 μl portion in 990 μl of 0.1% SDS solution and measuring the absorbance at 260 and 230 nm (Kalb et al., 1977). Equal numbers of nuclei from preparations from control, STZ-diabetic and fasted rats were allowed to complete the synthesis of nascent RNA transcripts in a reaction mixture containing 58.7 mM Hepes, pH 7.5, 80 mM KCl, 11.7 mM NaCl, 6.5 mM dithiothreitol, 5 mM $MgCl_2$, 78 μM EDTA, 78 μM EGTA, 0.6 mM ATP, 0.3 mM GTP, 0.3 mM CTP and 0.4 μM

[α-^{32}P]UTP (250 μCi/reaction) with 40 units of RNasin (RNAase inhibitor) in a total volume of 230 μl at 25 °C. After 30 min, the samples were subjected to DNAase digestion for 5 min at 25 °C by the addition of 25 units of RNAase-free DNAase followed by the addition of 3 ml of a solution containing 4 M guanidinium thiocyanate, 20 mM sodium acetate, pH 5.2, 0.5% *N*-laurylsarcosine and 5% (v/v) 2-mercaptoethanol. The ^{32}P-labelled RNA was isolated by centrifugation (150000 *g*; 35000 rev./min) through 5.7 M CsCl (1.5 ml) in a Beckman SW55 rotor (16 h, 18 °C).

The ^{32}P-labelled RNA was recovered by resuspending the pellet initially in 100 μl of 10 mM Tris/HCl, pH 7.4, buffer containing 5.0 mM EDTA and 1% SDS at 65 °C followed by three 100 μl washes with diethylpyrocarbonate-treated water. The RNA was recovered by ethanol precipitation and subjected to DNAase digestion for 30 min at 37 °C with 10 units of RNAase-free DNAase in 40 mM Tris/HCl, pH 7.6, buffer containing 6.0 mM $MgCl_2$, 10 mM NaCl, 1 mM dithiothreitol and 40 units of RNasin. The samples were extracted once with chloroform/butanol (4:1, v/v). To ensure complete denaturation of the transcripts and to increase hybridization, the ^{32}P-labelled RNA was partially hydrolysed in 0.2 M NaOH (15 min on ice) followed by neutralization with 1 M Hepes (pH 5.2) (Linial et al., 1985). The ^{32}P-labelled RNA was recovered by ethanol precipitation and resuspended in 1 ml of hybridization solution (see above) by heating at 65 °C for 10 min. The concentration of ^{32}P-labelled RNA was determined by liquid-scintillation spectrometry, and all samples being compared were adjusted to equal concentrations (c.p.m./ml) and volume (1 ml) with hybridization solution. Hybridization was carried out using Hybond-N to which 2 μg of several individual cDNAs of interest were fixed by u.v. cross-linking. Each cDNA was gel-purified from its plasmid vector after overnight digestion with the appropriate restriction enzyme and then denatured in 0.1 M NaOH for 30 min at 37 °C, neutralized in the presence of 10 × SSPE (1 × SSPE = 0.15 M NaCl, 0.01 M $NaHPO_4$, pH 7.4, and 1.0 mM EDTA), and applied to Hybond-N using a slot-blot apparatus (Mini-fold II, Schleicher and Schuell). All filters were prehybridized for 4 h at 47 °C in prehybridization solution (see above). Each filter for a given RNA preparation was trimmed, cut in two and placed back-to-back in a Seal-A-Meal bag (Dazey Corp.) to which the 1 ml of sample hybridization solution was added. This technique permitted the detection of as many as 14 different transcripts simultaneously. Hybridization was carried out for 2.5 days at 47 °C after which the filters were washed for 30 min at 50 °C in 2 × SSC, 30 min at 37 °C in 2 × SSC containing 10 μg/ml RNAase A, and for 30 min at 55 °C in 0.1 × SSC/0.1% SDS. After drying, the membranes were subjected to autoradiography with intensifying screens for 7–14 days. All bands were quantified by laser densitometry.

Statistical analyses

All data are expressed as means ± S.E.M. A one-way analysis of variance was performed on all groups with the Dunnett's test utilized to determine statistical differences. Significance was set at the $P < 0.05$ level.

RESULTS

In a pilot study, the relationship between oxidative capacity and the level of H-FABP was analysed in gastrocnemius muscles obtained from a previous study in which oxidative capacity had been increased by exercise training and/or treatment with β-guanidinopropionic acid (β-GPA). β-GPA is a drug that induces mitochondrial biogenesis and increases fatty acid-oxidative capacity in muscle. Muscles with a wide range of citrate synthase activities (Srere, 1969) were chosen without respect for the experimental group, and the level of H-FABP mRNA was measured. Figure 1 shows positive correlation ($r = 0.68$, $P < 0.05$) between citrate synthase activity and H-FABP mRNA levels, indicating that H-FABP mRNA levels parallel the increases in fatty acid-oxidative capacity in the muscle. These results led to further investigation of the expression of H-FABP during metabolic conditions that increase fatty acid oxidation. The significant, although low, correlation coefficient ($r = 0.68$,

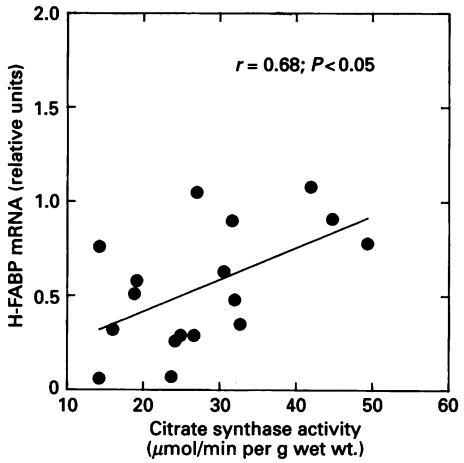

Figure 1 Correlation between H-FABP mRNA and citrate synthase activity in gastrocnemius muscles

Whole gastrocnemius muscles with a wide range of oxidative capacities taken from a previous experiment in which rats were exercise trained and/or treated with β-GPA were analysed for citrate synthase activity and H-FABP mRNA.

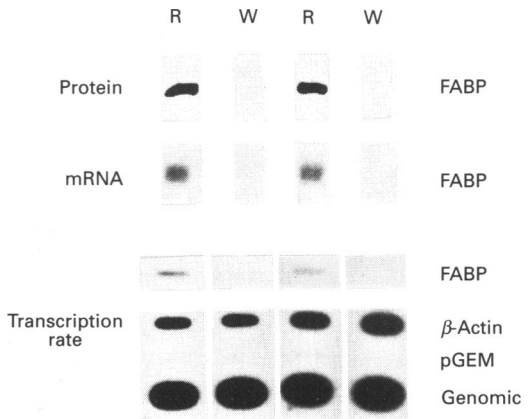

Figure 2 Autoradiograms demonstrating Western-blot, Northern-blot and run-on analyses of H-FABP in red and white quadriceps from control animals

Total protein and RNA were isolated from red and white portions of quadriceps and nuclei were isolated from red and white portions of gastrocnemius/plantaris and quadriceps from control rats. Each lane represents equal protein (20 μg of cytosolic protein), total RNA (10 μg), and nascent RNA. In the nuclear run-on analyses, pGEM (non-specific plasmid DNA) was used as a negative control, and all transcripts were normalized to genomic DNA to account for slight variations in RNA transcript concentration in the hybridization solution. R, red skeletal muscle; W, white skeletal muscle.

Table 1 Effect of STZ-induced diabetes and fasting on H-FABP protein, mRNA and transcription rate

Values are means ± S.E.M. relative to control ($n = 7$ for all three groups in the protein analysis, $n = 10$ for all three groups in the mRNA analysis, $n = 10$ for control and diabetic groups and $n = 4$ for the fasted group in the transcription analysis). * $P < 0.05$, compared with control.

	Control	Diabetic	Fasted
H-FABP	1.00 ± 0.28	1.80 ± 0.27*	1.93 ± 0.16*
H-FABP mRNA	1.00 ± 0.20	2.34 ± 0.14*	2.48 ± 0.12*
H-FABP transcription rate	1.00 ± 0.12	0.98 ± 0.17	2.17 ± 0.66

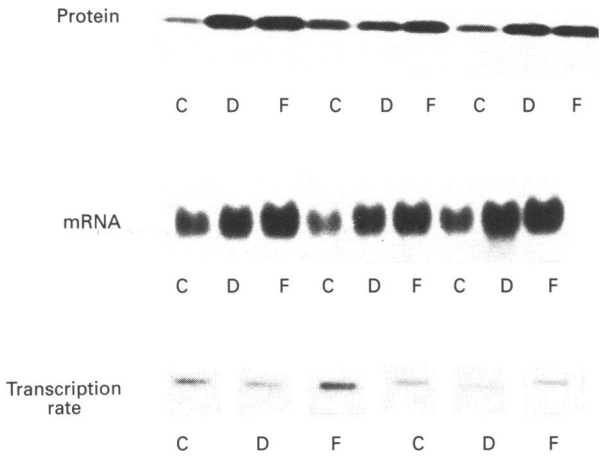

Figure 3 H-FABP concentration, mRNA concentration and transcription rate in red quadriceps from control, diabetic and fasted rats

Cytosolic proteins were isolated from red quadriceps from control ($n = 7$), 7-day STZ-diabetic ($n = 7$) and 3-day fasted ($n = 7$) rats. Protein (20 μg) was separated, transferred to Immobilon, and probed with an H-FABP antibody as described in the Materials and methods section. Total RNA was isolated from control ($n = 10$), 7-day STZ-diabetic ($n = 10$) and 3-day fasted ($n = 10$) rats. RNA (15 μg) was separated, transferred to Hybond-N and hybridized with an H-FABP cDNA probe. Nuclei were isolated from red gastrocnemius/plantaris and quadriceps from control ($n = 10$), 7-day STZ-diabetic ($n = 10$) and 3-day fasted ($n = 4$) rats, and subjected to run-on transcriptional analysis. C, control; D, 7-day STZ-diabetic; F, 3-day fasted.

$P < 0.05$) was probably due to the use of mixed muscle instead of pure red skeletal muscle.

To test the hypothesis that H-FABP is induced during conditions of increased fatty acid oxidation, male Sprague–Dawley rats were injected with 0.75 mg STZ/kg body weight, fasted for 3 days or maintained as controls. Red and white quadriceps were excised and freeze-clamped. Muscles were powdered and analysed for H-FABP and its mRNA content by Western- and Northern-blot analysis and H-FABP transcription rate by run-on analyses. Figure 2 shows representative autoradiograms of these three analyses, with each lane representing equal loads of protein, mRNA or nascent RNA transcripts from either red or white control skeletal muscle. Expression of H-FABP and its mRNA was high in red skeletal muscle compared with the nearly undetectable expression in white skeletal muscle. In agreement with the protein and mRNA data, transcription of the H-FABP gene was much greater in red than white skeletal muscle.

Table 1 summarizes the H-FABP, mRNA and transcription run-on data from red skeletal muscle of control, STZ-induced diabetic and fasted animals. Representative blots are shown in Figure 3. Both STZ-induced diabetes and fasting resulted in a 2-fold increase in H-FABP content. A similar trend was found for mRNA content in all three groups. Although the level of mRNA was much lower in white muscle, the overall trends were identical with those found in red muscle (results not shown).

In order to determine whether the increased levels of FABP mRNA associated with STZ-induced diabetes and fasting originated at the level of transcription of the H-FABP gene, run-on analyses were performed. Table 1 shows that there was no change in the transcription rate in red skeletal muscle in response to STZ-induced diabetes. However, fasting elicited a 2-fold increase in the transcription rate of the H-FABP gene. The effects of STZ-induced diabetes and fasting on the transcription rate in white muscle could not be measured accurately because of the nearly undetectable transcription rate.

DISCUSSION

The hypothesis that H-FABP is induced under conditions in which fatty acid oxidation has been shown to increase was tested under two conditions: STZ-induced diabetes and fasting in red and white skeletal muscle. Red skeletal muscle differs from white skeletal muscle in several ways, including higher capillary density, oxidative enzyme content and mitochondrial density. These inherent characteristics of red muscle account for its overall higher respiratory capacity and ability to oxidize fatty acids under both resting and exercise conditions. In contrast, white skeletal muscle is characterized by a low respiratory capacity and high glycogenolytic activity. If the function of H-FABP is to transport fatty acids to the mitochondria for subsequent oxidation, then it follows that red skeletal muscle should contain more H-FABP than white skeletal muscle. Figure 2 confirms this obvious difference in protein and mRNA levels between red and white control skeletal muscle.

An increase in mRNA can be caused by an increase in the transcription rate and/or an increase in mRNA stability. By measuring the transcription rate of the H-FABP gene in red and white skeletal muscle, we have shown that the greater amount of H-FABP mRNA in red skeletal muscle can at least in part be explained by a greater basal transcription rate.

To test the hypothesis that H-FABP expression is increased under metabolic conditions that increase fatty acid oxidation, rats were treated with STZ or were fasted for 3 days. Both of these conditions have previously been shown to increase fatty acid oxidation. The effect of STZ on H-FABP, its mRNA and transcription rate in skeletal muscle has not previously been reported. The effect of fasting on H-FABP of heart muscle was studied by Paulussen et al. (1989), who showed an insignificant decrease in tissue from fasted (66 h) animals compared with control. In contrast with the results of Paulussen et al. (1989), Table 1 shows that STZ-induced diabetes and fasting cause an increase in H-FABP and mRNA levels. These findings support the hypothesis that the synthesis of H-FABP is induced during periods of increased fat oxidation.

Although both STZ-induced diabetes and fasting elicit an increased expression of H-FABP, the two metabolic conditions do so by different mechanisms. The increased mRNA levels elicited by fasting are due, at least in part, to an increase in the transcription of the H-FABP gene. In contrast with these findings, STZ-induced diabetes does not increase the transcription rate of this gene. These results imply that at least two mechanisms of control can be employed in the regulation of H-FABP expression, one at the level of transcription and one possibly at the level of mRNA stability.

Unfortunately, the determination of H-FABP mRNA half-life in skeletal muscle *in vivo* is very difficult for several reasons. Because of the relatively low transcription rate of the H-FABP gene in skeletal muscle, and the extreme abundance of H-FABP mRNA in skeletal muscle reported by others (Claffey et al., 1987; Heukeroth et al., 1987; Sarzani et al., 1988) and confirmed by our laboratory, the turnover rate of the H-FABP message is probably extremely low. Extended exposure times to transcriptional inhibitors would allow phenomena such as extended mRNA half-lives (Endo et al., 1973) and superinduction (Tomkins et al., 1972) to interfere with an accurate measurement of the half-life. The complications caused by transcriptional inhibitors injected into whole animals limits the type of messages for which turnover rate can be accurately determined and certainly restricts our ability to study the effects under altered metabolic conditions such as STZ-induced diabetes. Because of the difficulties encountered in measuring mRNA half-lives in whole animals, most studies utilize cell culture systems. As for H-FABP, we have been unsuccessful in discovering a skeletal muscle cell line that contains H-FABP.

In conclusion this study has shown that under conditions that have previously been shown to induce fatty acid oxidation, namely STZ-induced diabetes and fasting, the concentration of H-FABP and its mRNA increased 2-fold. In addition, in the case of red skeletal muscle compared with white muscle, and the fasted group, the increase in mRNA can be explained, at least in part, by an increase in the rate of transcription. However, in the case of STZ-induced diabetes, an increase in transcription rate cannot account for the increase in H-FABP mRNA. These results imply that the expression of H-FABP in skeletal muscle can be regulated at several levels including transcriptional activation and probably mRNA stability. The details of these unknown mechanisms of control are topics for further investigations.

We gratefully acknowledge Dr. Jeffrey Gordon for his gift of the H-FABP cDNA. This research was supported by grant RO1 DK38416.

REFERENCES

Bass, N. M. (1988) Int. Rev. Cytol. **111**, 143–184
Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
Claffey, K. P., Herrera, V. L., Brecher, P. and Ruiz-Opazo, N. (1987) Biochemistry **26**, 7900–7904
Cornelius, P., Marlowe, M., Lee, M. D. and Pekala, P. H. (1990) J. Biol. Chem. **265**, 20506–20516
Crisman, T. S., Claffey, K. P., Saouaf, R., Hanspal, J. and Brecher, P. (1987) J. Mol. Cell Cardiol. **19**, 423–431
Endo, Y., Seno, H., Tominaga, H. and Natori, Y. (1973) Biochim. Biophys. Acta **299**, 114–120
Feinberg, A. P. and Vogelstein, B. (1983) Anal. Biochem. **132**, 6–13
Glatz, J. F. C., Van der Vusse, G. J. and Veerkamp, J. H. (1988) New. Physiol. Sci. **3**, 41–43
Glatz, J. F. C., Meyers, J., Van Enckevort, E., Vork, M. M. and van der Vusse, G. J. (1991) FASEB J. **5A**, 1641
Heuckeroth, R. O., Birkenmeier, E. H., Levin, M. S. and Gordon, J. I. (1987) J. Biol. Chem. **262**, 9709–9717
Hjerten, S. (1962) Arch. Biochem. Biophys. **99**, 466–467
Kalb, V. F., Jr. and Bernlohr, R. H. (1977) Anal. Biochem. **82**, 362–371
Linial, M., Gunderson, N. and Groudine, M. (1985) Science **230**, 1126–1132
Masoro, E. J. (1967) J. Biol. Chem. **242**, 1111–1114
Miller, W. C., Hickson, R. C. and Bass, N. M. (1988) Proc. Soc. Exp. Biol. Med. **189**, 183–188
Ockner, R. K., Kaikaus, R. M. and Bass, N. M. (1992) New Developments in Fatty Acid Oxidation (Coates, P. M. and Tanaka, K., eds.), pp. 189–204, Wiley–Liss, New York
Paulussen, J. A., Geelen, M. J. H., Beynen, A. C. and Veerkamp, J. H. (1989) Biochim. Biophys. Acta **1001**, 201–209
Paulussen, J. A., Van Moerkerk, H. T. B. and Veerkamp, J. H. (1990) Int. J. Biochem. **22**, 393–398
Peeters, R. A., In't Groen, M. A. and Veerkamp, J. H. (1989) Arch. Biochem. Biophys. **274**, 556–563
Peeters, R. A., Veerkamp, J. H., Van Kessel, A. G., Kanda, T. and Ono, T. (1991) Biochem. J. **276**, 203–207
Puissant, C. and Houdebine, L. (1990) Biofeedback **8**, 148–149
Sarzani, R., Claffey, K. P., Chobanian, A. V. and Brecher, P. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7777–7781
Schagger, H. and von Jagow, G. (1987) Anal. Biochem. **166**, 368–379
Schein, P. S., Alberti, K. G. M. M. and Williamson, D. H. (1971) Endocrinology **89**, 827–834
Srere, P. A. (1969) Methods Enzymol. **13**, 3–5
Tomkins, G. M., Levinson, B. B., Baxter, J. D. and Dethlefsen, L. (1972) Nature (London) New Biol. **239**, 9–14
Van Breda, E., Keizer, H. A., Vork, M. M., Surtel, D. A. M., de Jong, Y. F., van der Vusse, G. J. and Glatz, J. F. C. (1992) Pflügers Arch. **421**, 274–279
Veerkamp, J. H. and van Moerkerk, H. T. B. (1993) Mol. Cell. Biochem. **123**, 101–106
Veerkamp, J. H., Peeters, R. A. and Maatman, R. G. H. J. (1991) Biochim. Biophys. Acta **1081**, 1–24
Veerkamp, J. H. and van Moerkerk, H. T. B. (1992) in New Developments in Fatty Acid Oxidation (Coates, P. M. and Tanaka, K. eds.), pp. 205–210, Wiley–Liss, New York
Watanabe, M., Ono, T. and Kondo, H. (1991) J. Anat. **174**, 81–95
Zahradka, P., Larson, D. E. and Sells, B. H. (1989) Exp. Cell Res. **185**, 8–20

Received 4 May 1993/13 September 1993; accepted 4 October 1993