Exercise Down-Regulates Hepatic Fatty Acid Synthase in Streptozotocin-Treated Rats

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ABSTRACT An acute bout of prolonged exercise has been shown to decrease hepatic fatty acid synthase (FAS) mRNA and activity induced by high carbohydrate diets. The purpose of the current study was to examine the role of insulin in this exercise down-regulation of FAS. Sixty-four male Wistar rats were randomly divided into normal and streptozotocin (STZ)-treated diabetic groups. After being starved for 48 h and refed a high cornstarch (C) or fructose (F) diet for 10 h, one half of each group of rats was killed after an acute bout of prolonged exercise (E), while the other half of the group was killed in the rested state. STZ treatment suppressed plasma insulin and elevated plasma glucagon levels along with a severe hyperglycemia. FAS mRNA levels decreased by 60% ($P < 0.05$) with STZ treatment but were 250% higher in F-fed versus C-fed rats. E abolished F-induced FAS mRNA levels in both normal and STZ rats and decreased plasma glucose concentration in STZ rats ($P < 0.05$). F-fed normal rats showed twofold higher hepatic FAS activity than did C-fed normal rats and this dietary induction was abolished by STZ ($P < 0.05$). FAS activity in normal rats was not affected by E and was increased with E in STZ rats. Nuclear protein binding to the insulin response sequence was not affected by STZ or diet and increased with E ($P < 0.05$). Carbohydrate response element binding was greater with F- versus C-feeding ($P < 0.05$) but unaffected by E. E enhanced inverted CCAAT-box element binding regardless of diet and STZ. We conclude that although insulin status had a great influence on FAS gene expression, E-induced down-regulation of FAS mRNA was not mediated by altered insulin response sequence binding but primarily by increased inverted CCAAT-box element binding to the FAS promoter and/or decreased concentration of carbohydrate metabolites. J. Nutr. 131: 2252-2259, 2001.

KEY WORDS: exercise • fatty acid synthase • fructose • insulin • transcription factor • rats

In mammalian species, high carbohydrate (CHO)$^1$/low fat diets result in de novo lipogenesis in the liver and adipose tissue (1). This is caused primarily by the induction of lipogenic enzymes, including fatty acid synthase (FAS), the key regulatory enzyme for hepatic fatty acid synthesis (1–3). The CHO-induced up-regulation of FAS is mediated by both metabolic and hormonal mechanisms (4). Insulin is the major hormone promoting hepatic lipogenesis via the stimulation of gene expression of FAS and several other lipogenic enzymes primarily by transcriptional activation, although alterations in FAS mRNA stability may also play a role (4,5). The stimulatory effect of insulin has been shown to result from the binding of upstream stimulatory factors to the insulin response sequence (IRS/A) in the promoter region of the FAS gene (6,7). Furthermore, hepatic FAS gene has been reported to contain an inverted CCAAT-box element (ICE) located adjacent to IRS/A. This region can bind the transcription factor NF-Y in response to cAMP and its occupancy can attenuate up-regulation of FAS by insulin in vitro (8).

Although CHO feeding has consistently been shown to induce hepatic lipogenic enzymes, fructose (F) ingestion in particular results in a greater effect than glucose or complex CHO, such as cornstarch (C), despite a lower insulin response (3,9–12). Therefore, CHO induction of FAS must be attributed at least partially to mechanisms independent of insulin. For example, the CHO response element (ChoRE) found within the first intron of the FAS gene has sequence similarity to known glucose/CHO response elements in other lipogenic and glycolytic genes and its binding has been shown to increase with a high F diet (13,14).

Endurance exercise causes various hormonal and metabolic changes that alter the cellular milieu in a number of tissues, including liver (15–17). Prolonged exercise consistently results in a decrease in insulin secretion and an elevation of plasma glucagon and catecholamine levels, both of which could decrease lipogenesis (8,19). In addition, endurance exercise decreases liver glycogen and blood glucose levels, which could attenuate lipogenic precursors and glycolytic metabolites in the liver. We have previously reported that an acute bout of exercise suppresses induction of both FAS mRNA and enzyme

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Abbreviations used: C, cornstarch; CHO, carbohydrate; ChoRE, carbohydrate response element; E, exercised; F, fructose; FAS, fatty acid synthase; ICE, inverted CCAAT-box element; IRS/A, insulin response sequence; R, rested; SREBP, sterol regulatory element binding protein; STZ, streptozotocin.
activity normally observed in rats starved and refed a high CHO diet (3). We also demonstrated that exercise attenuated nuclear protein binding to the IRS/A and ChoRE of the FAS gene in liver extracts, which was postulated to be partially responsible for the down-regulation of FAS enzyme activity (20). However, the effect of exercise on ICE binding has never been examined.

In the current study we investigated whether the exercise-induced down-regulation of FAS was due to a decrease in plasma insulin concentration and subsequent attenuation of the binding of IRS/A in rat liver. Liver nuclear binding to ChoRE and ICE in response to hormonal changes, diet and exercise was also investigated. To accomplish this goal, we used a streptozotocin (STZ)-treated rat model of type 1 diabetes in conjunction with high F or high C feeding to separate dietary and hormonal effects on FAS induction.

MATERIALS AND METHODS

Animals. Sixty-four male Wistar rats (age, 2 mo; body weight, ~150 g; Harlan Sprague-Dawley, Madison, WI) were used in the current study. The rats were housed individually in the animal facilities at the University of Wisconsin-Madison, using a reverse 12-h light/dark cycle (7:00–19:00 dark; 19:00–7:00 light) and consumed a nonpurified diet (Ralston-Purina, St. Louis, MO) and tap water ad libitum. The Animal Use Protocol was approved by the University of Wisconsin-Madison Biomedical Research Animals and Resource Center Review Committee. After a 1-wk acclimation period, rats were randomly divided into either STZ-treated (n = 32) or normal controls (n = 32).

Streptozotocin treatment. STZ rats were injected intraperitoneally with three doses of STZ (10 mg/kg body), dissolved in citric acid (pH 4.5) and injected for 15 min and plasma was stored at ~8°C until hormone and plasma glucose assays were performed.

Plasma glucose and hormonal analyses. Plasma glucose concentration was measured using a glucose oxidase assay (Sigma Diagnostics, St. Louis, MO). Plasma glucagon and insulin were determined using RIA kits obtained from Diagnostic Products (Los Angeles, CA) and Linco Research (St. Louis, MO), respectively.

Northern blot analysis. Total RNA was isolated from livers by the method of Chomczynski and Sacchi (22) with Trizol Reagent (Gibco Life Technologies, Gaithersburg, MD). Nucleic acid concentration was estimated by absorbance at 260 nm. RNA quality was examined with gel electrophoresis and ethidium bromide staining. Pilot experiments were performed to determine optimal RNA conditions. In most cases, 15 μg total RNA per lane was loaded onto a 0.8% agarose/formaldehyde gel and run at low voltage (~20 h) to facilitate separation. The gel was then soaked in 10X SSC to remove formaldehyde and in 0.05 mol/L NaOH (20 min) to facilitate transfer, and covalently bound to the filter by ultraviolet cross-linking (Stratagene, La Jolla, CA).

The cDNA probes for FAS and 18s were labeled with [α-32P]dCTP using random primer extension (23) with a labeling kit (Megaprep, Amersham, Arlington Heights, IL). Northern blots were prehybridized at 42°C overnight in a solution consisting of 50% formamide, 5X Denhardt’s solution, 5X SSPE and 0.1% SDS. Radiolabeled probes were added at a level of 33 MBq/L hybridization solution and allowed to hybridize overnight. The stringency washes consisted of two 20-min washes with 1X SSC; 0.5% SDS at 45°C and two 20-min washes with 0.3X SSC; 0.5% SDS at 50°C, and one 20-min wash with 0.1X SSC; 0.5% SDS at 60°C. Filters were wrapped in plastic while still damp and exposed to film at ~80°C. After autoradiography, the probe was stripped from filters with an 80°C wash of 50% formamide, 2X SSPE at 65°C for 60 min.

FAS signals were quantified using a scanning densitometer (Bio-Rad model GS-670; BioRad, Richmond, CA). FAS values were expressed relative to the densities of the respective 18s values.

Gel mobility shift assays. Nuclear extracts were prepared as by the method of Dignam et al. (24) with modifications by Andrews and Fallar (25). The following single-stranded oligonucleotides were obtained from Gibco Life Technologies.

FAS-IRES-A (-71/-50): TCACGGCCATGTGGGGTGCCCGC
FAS-ChoRE (+283/+303): GGGCGCCTGTCACGTGGGGGCC
FAS-ICE (-109/-85): CGACGGCTCATGGGCTGGGCG

Each single-stranded probe was 5′-end-labeled by incubating with [γ-32P]dATP and T4 polymerase kinase (Promega, Madison, WI). The labeling reaction (25 μL) was allowed to proceed for 30 min at 37°C and stopped by adding 0.5 μmol/L EDTA and bringing the probe to a volume of 100 μL, with 1X TE buffer (100 mmol/L Tris-Cl, pH 8.0, and 10 mmol/L EDTA). After chloroform extraction, free [γ-32P]dATP was removed by spin chromatography and then ethanol precipitation. Complementary labeled stands were annealed by combining equal amounts of each oligonucleotide in TE buffer (pH 8.0), heating to 90°C and allowing to cool slowly to room temperature.

All binding reactions were performed at room temperature in 30 μL; however, conditions for the reactions were dependent on the oligonucleotide probe. For FAS-IRES-A: 10 mmol/L Tris HCl (pH 8.0), 50 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 10% glycerol and 0.5 μg poly[d(I-C)]. For FAS-ChoRE: 10 mmol/L Hepes (pH 8.0), 50 mmol/L NaCl, 50 mmol/L KCl, 5 mmol/L MgCl2, 2 mmol/L DTT, 17.5% glycerol and 1 μg poly[d(I-C)]. For FAS-ICE: 20 mmol/L Hepes (pH 7.9), 1 mmol/L EDTA, 60 mmol/L KCl, 0.5 mmol/L EDTA, 5% glycerol and 30 mg/mL poly[d(I-C)]. Each reaction contained ~1.5 MBq (0.1–0.5 ng) of radiolabeled oligonucleotides and the indicated amounts of nuclear extracts in a total volume of 30 μL. For competition experiments, unlabeled competitor DNA was added to the mixture before addition of labeled probe. After 20 min at room temperature, the samples were subjected to 4% nonreducing PAGE in 1X TG buffer (25 mmol/L Tris-Cl and 250 mmol/L glyc
cine). The dried gels were exposed to X-ray film at $-70^\circ$C with an intensifying screen.

**Enzyme assays.** Maximal activity of FAS was measured in liver cytosol according to Nepokroeff et al. (26). Briefly, frozen liver was homogenized in phosphate bicarbonate buffer [70 mmol/L KHCO$_3$, 85 mmol/L K$_2$HPO$_4$, 9 mmol/L KH$_2$PO$_4$ and 1 mmol/L DTT (pH 8.0)]. The homogenate was centrifuged at 20,000 $\times$ g for 10 min and the supernatant was recentrifuged at 105,000 $\times$ g for 60 min. Enzyme activity was measured spectrophotometrically by following the oxidation of NADPH at 340 nm for 3 min at 30°C. Protein content was determined by the Bradford method (27) with bovine serum albumin as the standard.

**Statistical analyses.** Three-way ANOVA was used to determine significant differences ($P < 0.05$) in means among the various treatment groups. The main effects are diet (F vs. C), exercise (E vs. R) and insulin status (diabetics vs. normal). When the overall F test was significant, a post hoc least significant difference test (SYSTAT Inc., Evanston, IL) was used.

**RESULTS**

**Body weight, food consumption and running time.** Body weight before the starvation/refeeding regimen was significantly less in STZ-treated rats than in normal rats ($P < 0.05$; Table 1). After 10 h refeeding, body weight remained lower in STZ versus normal rats ($P < 0.05$) and C- and F-fed rats did not differ. Food intake, expressed both as absolute and relative values (g/100 g body) during the refeeding period was significantly higher in F-fed rats ($P < 0.05$). Furthermore, STZ rats had lower absolute food intake than normal rats ($P < 0.05$), but their relative food intake was not different from that of the normal rats.

Running times of the various treatment groups did not differ.

**Plasma glucose.** Rats treated with STZ showed clear hyperglycemia ($P < 0.001$; Table 2). STZ treatment increased plasma glucose 1- to 2-fold in the R rats but no significant difference was observed between C- and F-fed rats. E resulted in a significantly lower plasma glucose concentration in STZ-treated rats but not in normal rats ($P < 0.05$, STZ $\times$ E interaction).

**Plasma insulin and glucagon.** Plasma insulin concentration was decreased with both STZ treatment ($P < 0.05$) and E ($P < 0.05$; Table 2). STZ-treated rats had significantly higher plasma levels of glucagon than normal controls ($P < 0.05$, main effect only). Diet and exercise did not significantly affect plasma glucagon status. STZ treatment resulted in a higher glucagon:insulin ratio than normal controls ($P < 0.05$). Furthermore, E tended to increase this ratio across all groups ($0.05 < P < 0.1$).

**Fatty acid synthase mRNA levels.** Northern analysis was performed on hepatic total cellular RNA and major transcripts were detected corresponding to 9.0 and 8.6 kb (Fig. 1A). The blot also depicts the relative abundance of 18S RNA, used as a reference for data normalization. F-fed R normal rats had a 2.5-fold higher FAS mRNA abundance ($P < 0.05$) compared with their C-fed counterparts (Fig. 1b). STZ treatment suppressed FAS mRNA levels by > 60% ($P < 0.05$) in both C- and F-fed rats; however, F-fed, STZ-treated rats still had significantly higher FAS mRNA abundance than did C-fed STZ rats in the R state ($P < 0.05$). Liver FAS mRNA levels were significantly decreased by E ($P < 0.05$). E decreased FAS mRNA levels by 65% and 40% ($P < 0.05$) in F-normal and STZ-treated rats, respectively. Thus, E completely abolished FAS induction caused by F feeding. Furthermore, this exercise effect was not influenced by insulin status, i.e., F induction of FAS mRNA in STZ-treated rats was also significantly attenuated with E.

**Transcription factor binding.** The specificity of nuclear protein binding to IRS/A, ICE and ChoRE was determined by the competition gel mobility shift assays. A single major DNA-protein complex was detected with labeled FAS-IRS/A blot also depicts the relative abundance of 18s RNA, used as a reference for data normalization. F-fed R normal rats had a 2.5-fold higher FAS mRNA abundance ($P < 0.05$) compared with their C-fed counterparts (Fig. 1b). STZ treatment suppressed FAS mRNA levels by > 60% ($P < 0.05$) in both C- and F-fed rats; however, F-fed, STZ-treated rats still had significantly higher FAS mRNA abundance than did C-fed STZ rats in the R state ($P < 0.05$). Liver FAS mRNA levels were significantly decreased by E ($P < 0.05$). E decreased FAS mRNA levels by 65% and 40% ($P < 0.05$) in F-normal and STZ-treated rats, respectively. Thus, E completely abolished FAS induction caused by F feeding. Furthermore, this exercise effect was not influenced by insulin status, i.e., F induction of FAS mRNA in STZ-treated rats was also significantly attenuated with E.

**TABLE 1**

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<tr>
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<tr>
<td>Before starvation, g</td>
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<tr>
<td>Rested</td>
<td>158.1 ± 5.1</td>
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<td>162.6 ± 3.7</td>
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<tr>
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<td>Rested</td>
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<td>5.7 ± 0.9</td>
<td>10.4 ± 0.5†</td>
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<td>7.9 ± 0.6</td>
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<td>9.6 ± 0.3</td>
<td>8.8 ± 0.3†</td>
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<td><strong>Relative food intake</strong></td>
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<td>During refeeding, g/100 g body</td>
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<tr>
<td>Rested</td>
<td>5.7 ± 0.4</td>
<td>5.1 ± 0.8</td>
<td>7.8 ± 0.3†</td>
<td>8.1 ± 0.6†</td>
</tr>
<tr>
<td>Exercised</td>
<td>6.1 ± 0.4</td>
<td>5.5 ± 0.7</td>
<td>7.6 ± 0.3†</td>
<td>7.8 ± 0.3†</td>
</tr>
<tr>
<td><strong>Running time, min</strong></td>
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</tr>
<tr>
<td>Rested</td>
<td>—</td>
<td>125 ± 52</td>
<td>173 ± 21</td>
<td>130 ± 60</td>
</tr>
<tr>
<td>Exercised</td>
<td>168 ± 35</td>
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Values are means ± SEM, $n = 8$. # $P < 0.05$, STZ vs. normal within each dietary treatment. † $P < 0.05$, F vs. C within normal or STZ. * $P < 0.05$, exercised vs. rested within each diet and STZ treatment.
EXERCISE INHIBITION OF FATTY ACID SYNTHASE

TABLE 2

Plasma glucose, insulin and glucagon concentrations in rested or exercised normal and diabetic rats fed C or F

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<th>Normal</th>
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<th>STZ</th>
<th>ANOVA P &lt; 0.05</th>
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<td>Rested</td>
<td>8.7 ± 1.7</td>
<td>18.1 ± 2.3</td>
<td>8.2 ± 0.6</td>
<td>26.4 ± 1.2</td>
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<td>14.1 ± 4.8</td>
<td>11.8 ± 1.8</td>
<td>9.0 ± 0.3</td>
<td>17.6 ± 1.9</td>
<td>STZ × E</td>
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<td>Insulin, pmol/L</td>
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<td>Rested</td>
<td>125.2 ± 18.1</td>
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<td>177.6 ± 29.9</td>
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<td>77.7 ± 8.2</td>
<td>34.1 ± 4.8</td>
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<tr>
<td>Rested</td>
<td>64.1 ± 7.9</td>
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<td>63.1 ± 12.4</td>
<td>70.2 ± 6.5</td>
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<tr>
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<td>73.3 ± 9.9</td>
<td>49.8 ± 9.7</td>
<td>67.3 ± 3.9</td>
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<td>Glucagon/insulin</td>
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<td>Rested</td>
<td>0.69 ± 0.23</td>
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<td>0.39 ± 0.06</td>
<td>2.00 ± 0.51</td>
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<tr>
<td>Exercised</td>
<td>0.89 ± 0.25</td>
<td>2.07 ± 0.59</td>
<td>0.74 ± 0.21</td>
<td>2.40 ± 0.48</td>
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Values are means ± SEM, n = 8, *P < 0.05, STZ vs. normal within each dietary treatment. †P < 0.05, F vs. C within normal or STZ. *P < 0.05, exercised (E) vs. rested within each diet and STZ treatment.

ICE or a consensus NFκB oligonucleotide probe did not affect binding to the labeled IRS/A (Fig. 2A, panel A, lanes 6–16), indicating that the band detected was specific. To determine whether the binding of transcription factors was affected by nutritional and metabolic factors, liver nuclear extracts were prepared from pooled nuclear extracts from each treatment group (Fig. 2A, panel B). Diet and STZ treatment had no effect on IRS/A binding (Fig. 2B). However, IRS/A binding was increased overall by exercise (main effect, P < 0.05).

Incubation of nuclear proteins with FAS-ICE produced two major complexes, both of which disappeared with the competition of excess unlabeled ICE at concentrations >10-fold molar excess (Fig. 3A, panel A, lanes 1–5). Addition of excess NFκB oligonucleotide did not result in competition with ICE (panel A, lanes 11–15). Although addition of unlabeled IRS/A at low concentration (1- to 10-fold) did not compete with ICE, a 50- to 100-fold molar excess of IRS/A did result in disappearance of the lower major band (panel A, lanes 9–10).

It remains to be elucidated whether this competition between IRS/A and ICE binding was simply an experimental artifact or revealed an unknown mechanism. Gel shift experiments with nuclear extracts pooled from each treatment group is shown in Figure 3A (panel B). When individual gels were analyzed, we observed no diet or STZ treatment effect on ICE binding; however, ICE binding was significantly higher in nuclear extracts from E than from R rats (P < 0.05, main effect; Fig. 3B).

Competition experiments with FAS-ChoRE were performed to determine the specificity of transcription factor binding (Fig. 4A). A single band representing DNA-protein complex was detected (panel A, lane 1). This complex disappeared due to competition with increasing concentrations of unlabeled ChoRE oligonucleotides (panel A, lanes 2–5), whereas adding increasing concentrations of unlabeled consensus NFκB oligonucleotide probes did not affect ChoRE binding (panel A, lanes 7–12), indicating that the band was specific. Patterns of ChoRE binding in the treatment groups are shown in Figure 4A (panel B), F feeding significantly increased nuclear protein binding to FAS-ChoRE (P < 0.05, main effect; Fig. 4B). STZ and E had no effect on the binding of this DNA sequence in rat liver nuclear extracts.

Fatty acid synthase activity. Refeeding fructose resulted in a twofold increase in FAS enzyme activity above levels found in C-fed rats regardless of exercise status (Fig. 5). STZ
treatment abolished this increase in F-fed rats ($P < 0.05$), but had no significant effect in C-fed rats. E did not affect FAS activity in C- or F-fed normal rats but resulted in an increase in FAS activity in STZ-treated rats ($P < 0.05$).

**DISCUSSION**

Our previous work has demonstrated that both an acute bout of prolonged exercise and chronic training can down-regulate hepatic FAS activity and mRNA levels in rats starved and refed a high CHO diet (3,12). We have also shown that decreased nuclear protein binding to IRS/A and/or ChoRE on the FAS gene in the early stages of refeeding (6 h) may play an important role in the exercise-induced down-regulation of FAS (20). Thus, we hypothesized in the current study that diminished plasma insulin status due to exercise could lead to a reduction of IRS/A binding and, hence, decrease hepatic FAS expression. To accomplish this goal, we used the STZ-diabetic rat model with starvation/refeeding of either a high C or F diet, in an attempt to separate hormonal and dietary effects on FAS induction.

STZ treatment severely hampered FAS mRNA expression in both diet groups and suppressed FAS activity in F-fed rats. These defects due to impaired insulin status were not completely corrected by fructose feeding, indicating that normal insulin status is required for the CHO-induced gene expression of FAS, at least with the starvation/refeeding rat model. Our data were consistent with a large body of literature demonstrating the essential role of insulin in diet-induced lipogenic enzyme induction (6,7). The insulin effect has been shown to be conferred by activated IRS/A binding in the promoter region of the FAS gene (6,7,28). Furthermore, this binding has been shown to involve upstream stimulatory factors (7,29,30). However, in the current study, IRS/A binding was not affected by STZ treatment, suggesting that impaired FAS gene expression in the diabetic rats is not caused by diminished IRS/A binding in the liver. Perhaps only a minimal amount of insulin...
is required to elicit sufficient IRS/A binding in diabetic rats, which supports the hypothesis that insulin plays only a permissive role in the lipogenic enzyme induction due to starvation and refeeding (6,7,28). However, the mechanism by which insulin regulates dietary induction of FAS is not entirely clear (18,19). Several recent studies have demonstrated that other transcription factors are involved in the insulin-mediated up-regulation of FAS gene expression (31,32). For example, the FAS promoter region contains an E-box motif (264/259) that was part of the well-defined IRS/A (271/250) and can bind with other basic helix-loop-helix transcription factors, such as adipocyte determination differentiation dependent factor 1/sterol regulatory element binding protein (SREBP) 1 to elicit transactivation of FAS (33). Moreover, transcription of adipocyte determination differentiation dependent factor 1/SREBP1 in hepatocytes was found to be controlled positively by insulin and negatively by glucagon and cAMP (34). Recently, it was observed in STZ-treated rats that one SREBP isofom (SREBP-1a) is unchanged in livers compared with normal controls, while another isofom (SREBP-1c) is decreased (31). In fact, the SREBP-1c:1a ratio is 96% lower in STZ-treated livers but returns to normal upon administration of insulin. These studies raise the possibility that the down-regulation of FAS induction during starvation/refeeding found in STZ-treated rats was caused by alterations in binding of other transcription factors to the FAS promoter not measured in the current study. However, the relative physiologic importance of the newly defined gene sequences remains to be determined.

STZ-treated rats had higher plasma glucagon concentration and an increased glucagon/insulin ratio, regardless of diet. As an anti-lipogenic hormone, glucagon blocks the increase in lipogenesis caused by either starvation/refeeding or insulin administration in rats (35,36). The inhibitory effect of glucagon may result from increased hepatic cAMP, which mimics glucagon in vitro (36,37). The importance of cAMP is highlighted by the fact that the FAS promoter region also contains ICE that can bind the transcription factor NPY (6,7,28). This region has been shown to be responsive to cAMP and its activation can attenuate up-regulation of FAS by insulin (8). Despite elevated plasma glucagon, ICE binding was not increased in STZ-treated rats. Thus, attenuated FAS expression in the diabetic rats might be mediated by some other mechanisms that were not revealed by the current study. For example, high levels of glycolytic metabolites are required to elicit FAS induction by CHO (18,19). Katsurada et al. observed 4.6- and 3.3-fold increases in FAS mRNA and enzyme activity, respectively, when diabetic rats were starved and refed fructose for 16 h (2). However, transcription rate increased only 60%, indicating FAS induction was mediated not by increased transcription, but enhanced stability of FAS mRNA. Whether the decreased FAS mRNA levels in STZ-treated rats was due to lower glycolytic intermediate concentrations in currently unknown.

F-fed rats showed 2.5-fold higher FAS mRNA and 2-fold higher FAS activity than C-fed rats. Furthermore, in the STZ-treated diabetic rats wherein FAS gene expression was heavily suppressed, FAS mRNA and activity were induced by F to a greater extent than by C and reached the same levels as
C-fed normal rats. These data, consistent with many previous studies, clearly demonstrated the prominent lipogenic potential of F (3,11,38–40). F-fed normal and diabetic rats consumed 40% and 60% more food than their C-fed counterparts, which could play an important role in explaining the greater FAS induction. However, the mechanism underlying the observed fructose effect cannot be entirely due to greater energy intake because previous studies using a pair-feeding or meal-feeding regimen revealed a greater lipogenic effect with fructose feeding despite isocaloric intake (3,39,40). FAS up-regulation by fructose feeding has been shown to coincide with increased IRS/A binding in rat and mouse liver (28,41). Therefore, a direct involvement of fructose in promoting FAS gene transcription was suggested. These studies were echoed by our recent experiment showing that refeeding a high F diet for 6 h to food-deprived rats significantly increased both IRS/A and ChoRE binding, along with a dramatic elevation (50-fold) of FAS gene transcription rate (20). In the present study, we observed an increased transcription factor binding to FAS-ChoRE in the F-fed rats, but IRS/A binding was not altered. There are two possible explanations for the discrepancy. There might have been a transient increase in IRS/A binding during the early phase of refeeding. By studying the time course of FAS induction by F, Katsurada et al. (2) revealed a peak FAS transcription rate at 4 h of refeeding, whereas FAS mRNA levels did not reach the maximal value until 16 h. Because increased nuclear protein binding is the overt expression of transactivation of FAS gene, we speculate that IRS/A binding might indeed have increased but then returned to normal at 9–10 h when rats were killed. Alternatively, F induction of FAS might have resulted primarily from increased mRNA stability, whereas increased transcription due to IRS/A and/or ChoRE binding might only play a minor role. F metabolites stimulate lipogenesis in rat liver more effectively than glucose (4). Incubating HepG2 cells with D-glucose, lactate and citrate, but not D-glucose, increased the half-life of FAS mRNA from 4.4 to 30 h, suggesting that the observed effects were mediated by glycolytic intermediates (5). In a previous study, we observed a greater hepatic pyruvate content in rats starved and refed a F diet for 12 and 24 h, which coincided with a greater FAS mRNA abundance (3). However, the importance of these potential regulatory mechanisms has not been confirmed under physiological conditions.

An acute bout of exercise significantly decreased F-induced hepatic FAS mRNA levels both in the normal and STZ-treated rats. We initially hypothesized that the exercise-induced down-regulation of FAS was caused by decreased plasma insulin and diminished nuclear protein binding to IRS/A and ChoRE. This hypothesis can be rejected based on the following findings. First, like F-fed rats, C-fed rats had lowered plasma insulin concentration with exercise but C-fed rats did not show decreased FAS mRNA or activity. Second, IRS/A binding was not decreased but instead increased with exercise. Third, ChoRE binding was not affected by exercise. Therefore, exercise-induced down-regulation of FAS is not directly caused by attenuated plasma insulin levels or by impaired IRS/A-ChoRE binding in the liver.

Exercise significantly increased nuclear protein binding to ICE, an inverted CCAT box located adjacent to IRS/A in the FAS promoter. This region has been shown to bind the transcription factor NF-Y in response to cAMP and its occupancy can attenuate up-regulation of FAS by insulin in vitro (8). Our finding suggests that ICE may play a more important role in FAS gene regulation during exercise than other gene regulatory sequences investigated so far. Although exercise simultaneously increased IRS/A and ICE binding in rat liver extracts, the magnitude of increase appeared to be greater for the latter in the F-fed rats (Fig. 3b), which might be sufficient to block the inductive effect of IRS/A and ChoRE. However, it is unclear why exercise can increase ICE binding in the liver. Plasma glucagon concentration was not elevated in exercised rats, possibly due to the milder work intensity in the current study, but other cAMP-generating hormones, such as catecholamines and vasopressin, could increase their plasma concentrations during prolonged exercise (21). Coupled with lower insulin, the hormonal milieu was in favor of producing high levels of hepatic cAMP that might activate ICE binding.

A major finding that has not been reported before is that exercise can decrease hepatic FAS mRNA levels in the STZ diabetic rats refed F despite severely impaired insulin status. In addition to the mechanisms mentioned above, these rats had decreased plasma glucose levels with exercise, raising the possibility that FAS mRNA in STZ-treated rats was less stable. F-fed diabetic rats and C-fed normal rats had similar FAS mRNA abundance in the resting state, but plasma insulin concentration in the former was only one third of the latter (Table 2), suggesting that diabetic rats rely heavily on mechanisms other than insulin to induce lipogenic enzymes upon feeding. Presumably, they have greater glycolytic intermediate levels that stabilize mRNA (18,19). An acute bout of exercise has previously been shown to decrease hepatic pyruvate and gluconeogenic precursors (16); thus, FAS mRNA in the STZ-treated rats could be more susceptible to degradation during prolonged exercise.

FAS enzyme activity was not down-regulated by exercise. This is not unexpected because the 10-h refeeding time was chosen primarily to manifest altered nuclear protein binding and mRNA transcription, whereas enzyme protein synthesis usually takes longer. In our previous study and others, peak FAS activity was reached at 24–48 h after the onset of refeeding (3). FAS activity measured at 10 h may still have been too low to show significant inhibition due to exercise.

In summary, we have shown for the first time that an acute bout of prolonged physical exercise could down-regulate hepatic FAS gene expression independent of rats’ insulin status. Exercise-induced down-regulation of FAS mRNA in both normal and diabetic rats was not caused by decreased plasma insulin level or IRS/A binding but coincided with increased binding of ICE. Diminished hepatic glycolytic intermediates during exercise might play a role in destabilizing FAS mRNA especially in the STZ-treated rats.

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LITERATURE CITED


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