Somatotropin-dependent decrease in fatty acid synthase mRNA abundance in 3T3-F442A adipocytes is the result of a decrease in both gene transcription and mRNA stability

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Somatotropin (ST) markedly decreases lipogenesis, fatty acid synthase (FAS) enzyme activity and mRNA abundance in pig adipocytes. The present study was conducted to determine whether the decrease in FAS mRNA in 3T3-F442A adipocytes was the result of a decrease in transcription of the FAS gene and/or a change in FAS mRNA stability. Insulin increased the abundance of FAS mRNA 2–13-fold and fatty acid synthesis 3–7-fold. Somatotropin decreased the stimulatory effect of insulin on the abundance of FAS mRNA and lipogenesis by 40–70% and 20–60% respectively. Subsequent run-on analyses demonstrated that the decrease observed in FAS mRNA in response to ST was associated with an 82% decrease in transcription; ST significantly shortened the half-life of FAS mRNA from 35 to 11 h. To corroborate the run-on analyses, cells were stably transfected with a pFAS–CAT5 (in which CAT stands for chloramphenicol acetyltransferase) reporter construct that contained 2195 bp of the 5′ flanking region of the rat FAS gene. Insulin treatment increased FAS–CAT activity 4.7-fold. When ST was added to the insulin-containing medium there was an approx. 60% reduction in FAS–CAT activity. In summary, our results indicate that ST decreases FAS mRNA levels and that this is the result of a marked decrease in both transcription of the FAS gene and stability of the FAS mRNA.

INTRODUCTION

A large number of studies have shown that treating growing pigs with pig somatotropin (ST) reduces adipose tissue accretion by as much as 60–80% while concurrently stimulating muscle growth by 40–60% (reviewed in [1]). Studies both *in vitro* and *in vivo* have shown that the suppression of adipose tissue growth by pig ST is due to a decrease in lipogenesis rather than an increase in lipolysis, and occurs because pig ST decreases adipocyte insulin sensitivity [1–4]. The pig ST-dependent decline in insulin sensitivity in pig adipocytes results in a decrease in glucose transport [2], the expression of insulin-stimulated key lipogenic enzyme genes including fatty acid synthase (FAS) [5–8] and an associated decrease in the activities of various lipogenic enzymes [2–8]. We have used the FAS gene as a model gene to learn more about how ST decreases lipogenesis and the stimulatory effects of insulin because FAS has a central role in lipogenesis *de novo* [9] and because transcription of the gene is exquisitely sensitive to nutritional and hormonal changes [10,11]. For example, insulin causes a marked increase in FAS mRNA levels that is the result of an increase in transcription of the gene, and fasting and refeeding modulate transcription of the gene markedly. Recently, we have shown that transcription of the FAS gene in rat liver is also strikingly affected by pig ST administration [7]. When rats were treated with pig ST there was an 80% decrease in transcription of the FAS gene [7]. Although this latter study demonstrated that transcription of the FAS gene in rat hepatocytes was decreased by ST, we are unaware of any studies that have addressed this question in adipocytes. Thus the present study was conducted to determine whether the ST-dependent decrease in FAS mRNA abundance in 3T3-F442A adipocytes was the result of a decrease in the transcription rate of the FAS gene or reflected changes in the stability of the mRNA.

EXPERIMENTAL

Materials

3T3-F442A preadipocytes were kindly provided by Dr. H. Green (Harvard University, Boston, MA, U.S.A.). Plasmid pcRFAS40, containing a 1.8 kb *Eco*RI–*Xho*I fragment of rat FAS cDNA, was kindly provided by Dr. M. Schweizer (AFRC Norwich Lab., Norwich, Norfolk, U.K.). Plasmid pMET-IG-mGHR, containing the mouse ST receptor cDNA gene, was kindly provided by Dr. J. Kopchick (Ohio University, Athens, OH, U.S.A.). Plasmid pDF8, containing a 1.06 kb *Bam*HI–*Eco*RI fragment of rat 18 S rRNA cDNA gene, was kindly provided by Dr. R. Torczynski (Cytoclonal Pharmaceutics, Dallas, TX, U.S.A.). Recombinant bovine ST (bST) was a gift from Monsanto Company (Dr. R. Ryan, St. Louis, MO, U.S.A.). Dulbecco's modified Eagle's medium (DMEM), geneticin and lipofectamine were purchased from Life Technologies (Grand Island, NY, U.S.A.). Sephadex G-50 spin columns were obtained from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Insulin was obtained from Collaborative Biomedical Products (Bedford, MA, U.S.A.). Calf serum and fetal bovine serum were purchased from Sigma (St. Louis, MO, U.S.A.). Genescreen, [α-^{32}P]dCTP and [α-^{32}P]UTP were from DuPont–NEN (Boston, MA, U.S.A.). Ready-To-Go random labelling kits were obtained from Pharmacia (Piscataway,

Abbreviations used: bST, bovine somatotropin; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco's modified Eagle's medium; FAS, fatty acid synthase; IRE, insulin response element; IRS, insulin receptor substrate; ST, somatotropin; USF, upstream stimulatory factor.

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NJ, U.S.A.). The pCAT-basic vector (in which CAT stands for chloramphenicol acetyltransferase), restriction enzymes and RNasin were purchased from Promega (Madison, WI, U.S.A.). All other chemicals were supplied by Sigma or Fischer Scientific (Pittsburgh, PA, U.S.A.).

Plasmid constructs

The pFAS–CAT5 plasmid was constructed by ligating the *Sal*I restriction fragment containing sequences from −2195 to +65 of the rat FAS gene into the *Sal*I cloning site of the promoterless pCAT-basic vector [12]. The insert sequences were verified by the dideoxy sequencing method [13].

Cell culture

3T3-F442A cells were maintained in DMEM with 10 % (v/v) calf serum in T-75 flasks at 37 °C. To convert the cells to adipocytes, preconfluent cells were incubated for 48 h in DMEM containing 10 % (v/v) fetal bovine serum and 2 μg/ml insulin [14]. After 48 h, insulin was removed and cells were fed every 2–3 days with DMEM plus 10 % (v/v) fetal bovine serum until 80 % of the cells were lipid-filled. Cells were cultured for 24 h in defined medium [DMEM plus 2 % (w/v) BSA, 5 μg/ml transferrin, 2 ng/ml selenium, 10 ng/ml α-tocopherol and 1 nM 3,3′,5-tri-iodothyronine] before the addition of insulin and/or bST. Insulin and bST were added to the above defined medium containing hydrocortisone (50 ng/ml) for the indicated periods of time. Defined medium plus hydrocortisone was the control medium for all experiments.

Lipogenesis assay

The lipogenic rate of cultured adipocytes was measured by quantifying the conversion of [^{14}C]glucose into lipids as described previously [15]. In brief, 1 μCi of [^{14}C]glucose was added to each flask of adipocytes treated with hormones for the indicated periods of time, then incubated for an additional 4 h period.

Northern blot analysis

Total RNA was extracted as described by Chomczynski and Sacchi [16]. Northern blotting analysis was conducted as described previously [6]. In brief, 15 μg of total RNA was separated by electrophoresis in a 1.0 % (w/v) agarose gel and transferred to Genescreen membranes. The RNA was cross-linked to the membrane by UV irradiation and baked for 2 h at 80 °C. Prehybridization and hybridization solutions contained 50 % (v/v) deionized formamide, 5 × SSPE [where SSPE is 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/1 mM EDTA], 5 × Denhardt's solution, 10 % (w/v) dextran sulphate, 1 % (w/v) SDS and 200 μg/ml salmon sperm DNA. Membranes were prehybridized overnight at 42 °C. The rat FAS cDNA (50 ng) was labelled with 50 μCi of [α-^{32}P]dCTP with the Ready-To-Go random labelling kit. Hybridization fluid containing approx. 10^6 c.p.m. of probe/ml was added and the membrane was hybridized for 24 h at 42 °C. Membranes were then washed twice for 5 min each at room temperature in 2 × SSC, twice for 30 min each at 65 °C in 2 × SSC/1 % (w/v) SDS and twice for 30 min each at room temperature in 0.1 × SSC. Membranes were then exposed to Kodak X-Omat AR film at −70 °C for approx. 24 h. Membranes were stripped in 0.1 × SSC and 1 % (w/v) SDS at 100 °C for 1 h and then reprobed with the cDNA for rat 18 S rRNA to assess variation in loading or transfer of RNA.

Nuclei isolation and nuclear run-on transcription assay

After 2 days of culture with the various hormone treatments, cells were rinsed once with ice-cold PBS and lysed in lysis buffer [10 mM Tris/HCl (pH 7.5)/5 mM $MgCl_2$/25 mM KCl/0.1 mM EDTA/1 mM dithiothreitol/0.5 % Nonidet P40]. Cells were homogenized by pipetting up and down. Nuclei were then collected by centrifugation at 500 ***g*** for 5 min and washed once with lysis buffer without Nonidet P40 and once with nuclei wash buffer [50 mM Tris/HCl (pH 7.8)/5 mM $MgCl_2$/0.1 mM EDTA/0.1 mM dithiothreitol]. Nuclei were resuspended in 100 μl of nuclei storage buffer [50 mM Tris/HCl (pH 7.8)/5 mM $MgCl_2$/0.1 mM EDTA/0.1 mM dithiothreitol/40 % (v/v) glycerol] and stored in liquid N_2. The run-on transcription assay was performed with a modification of the procedure described by Paulauskis and Sul. [17]. In brief, nuclei (approx. 10^7) were incubated with 200 μl of reaction mixture containing 5 mM Tris/HCl, pH 8.0, 2.5 mM $MgCl_2$, 0.15 M KCl, 40 units of RNasin, 1 mM each ATP, GTP, CTP and 80 μCi of [α-^{32}P]UTP at room temperature for 45 min. Nascent RNA species were then extracted with phenol/chloroform, co-precipitated with 5 μg of yeast RNA by adding 2 vol. of ethanol, and then resuspended in 200 μl of MilliQ water. The labelled RNA was then purified by elution over a G-50 spin column. Approx. 7×10^6 c.p.m./ml labelled RNA was hybridized with 2 μg of each linearized plasmid applied to Genescreen membranes by using a slot-blot manifold. The blots were washed and exposed to film as described above. Quantification of the signal was performed by densitometric scanning and the FAS signal was standardized with mouse ST receptor signal.

Stable transfections and CAT assay

The 3T3-F442A preadipocytes were grown in six-well plates in growth medium [DMEM plus 10 % (v/v) calf serum] until the cells were 60–80 % confluent. Chimaeric promoter construct (pFAS–CAT5) (2 μg) and pSV2-neo (40 ng) were diluted in 100 μl of antibiotic-free DMEM. The DNA solution was then added to 100 μl of lipofectamine solution (6 μl of lipofectamine plus 94 μl of antibiotic-free DMEM). After mixing gently, the DNA–lipofectamine complexes were incubated at room temperature for 20 min and were subsequently diluted in 800 μl of antibiotic-free DMEM and added to PBS-washed cells. After incubation with the DNA–lipofectamine complexes at 37 °C for 5 h, cells were cultured in antibiotic-free growth medium for 24 h before the addition of 800 μg/ml geneticin. After 2 weeks of geneticin selection, pools of geneticin-resistant cells were grown in the absence of geneticin and differentiated, then assayed for CAT expression after hormone treatments. The CAT assay was performed with [^{3}H]chloramphenicol by the method of Nordeen et al. [18]. In brief, adipocytes were lysed in 0.25 M Tris/HCl, pH 8.0, by four cycles of freezing and thawing. After heating at 60 °C for 10 min, the cell extract was incubated with 200 μl of the reaction mixture containing 1.5 μCi/ml [^{3}H]chloramphenicol, 0.3 mg/ml n-butyryl-CoA and 0.25 mM Tris/HCl, pH 8.0, at 37 °C for 17 h. The reaction mixture was then extracted with tetramethylpentadecane/xylenes (2:1, v/v), and radioactivity was determined with a β-counter. The CAT activity was normalized to protein content.

Statistical analysis

Results were analysed with the General Linear Model procedures of the Statistical Analysis System (SAS Institute, NC, U.S.A.), where significant differences were detected. Mean separation was

achieved by using T multiple comparison. All results are presented as means ± S.E.M. For the FAS mRNA stability assay, the percentages of mRNA results were transformed into logarithms of the percentage of mRNA and repeated-measures analysis of variance was used to determine the effect of treatment on the percentage of mRNA remaining.

RESULTS

Somatotropin suppresses the stimulatory effect of insulin on lipogenesis and FAS mRNA levels

Previously we have reported that treating growing pigs with pig ST can decrease adipose tissue FAS mRNA abundance by as much as 80 % [6]. In the present study we initially examined whether bST caused a similar effect in 3T3-F442A adipocytes. As shown in Figure 1, insulin increased FAS mRNA abundance approx. 7-fold. When cells were cultured with bST at 10, 100 and 1000 ng/ml in the presence of insulin, FAS mRNA abundance was decreased by 34 %, 65 % and 61 % respectively (Table 1). Thus these results establish that ST suppresses the stimulatory effect of insulin on FAS mRNA levels in 3T3-F442A adipocytes in a dose-dependent manner quite similar to that observed previously for pig adipocytes.

Previous studies have shown that pig ST treatment decreases

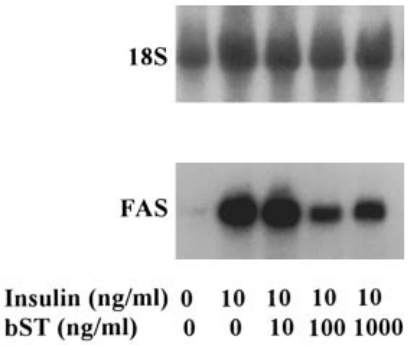

Figure 1 Effects of bST on insulin regulation of FAS mRNA abundance in 3T3-F442A adipocytes

3T3-F442A adipocytes were cultured for 48 h with insulin (10 ng/ml) and different doses of bST (10, 100 or 1000 ng/ml). Total RNA (15 μg) was hybridized with the FAS cDNA probe as described in the Experimental section. A representative blot from four independent experiments is shown.

Table 1 Effects of bST on insulin regulation of FAS mRNA abundance in 3T3-F442A adipocytes

3T3-F442A adipocytes were cultured for 48 h with insulin (10 ng/ml) and different doses of bST (10, 100 or 1000 ng/ml). FAS mRNA levels were quantified by scanning of autoradiographic signals from Northern blots and normalized with 18 S rRNA to account for differences in loading. Values are means ± S.E.M. for four independent experiments. Means with different superscripts are significantly different ($P < 0.05$).

Treatment	FAS mRNA levels (%) of control
Control	100^{a}
Insulin (10 ng/ml)	767 ± 78^{b}
Insulin + bST (10 ng/ml)	508 ± 142^{bc}
Insulin + bST (100 ng/ml)	272 ± 104^{ac}
Insulin + bST (1000 ng/ml)	300 ± 101^{bc}

Table 2 Temporal profile of the effects of insulin and bST on lipogenesis in 3T3-F442A adipocytes

Adipocytes were cultured in defined media with the indicated concentrations of insulin and bST for 24, 48 or 72 h. Incorporation of [^{14}C]glucose into lipid was determined as described in the Experimental section. The basal lipogenic rates of control cells at 24, 48 and 72 h were 45, 56 and 71 nmol of glucose incorporated into lipid per 4 h per mg of lipid respectively. Values are means ± S.E.M. for four independent experiments. Means with different superscripts within a time period are significantly different ($P < 0.05$).

	Lipogenesis (% of control)		
Treatment Time (h) ...	24	48	72
Insulin	429 ± 100^{a}	351 ± 61^{ab}	657 ± 210^{a}
Insulin + bST (10 ng/ml)	459 ± 76^{a}	469 ± 162^{b}	460 ± 123^{ab}
Insulin + bST (100 ng/ml)	329 ± 69^{a}	225 ± 23^{ab}	216 ± 36^{b}
Insulin + bST (1000 ng/ml)	323 ± 73^{a}	212 ± 33^{a}	247 ± 25^{b}

FAS enzyme activity markedly in pig adipose tissue [2,8]. To examine whether ST decreased the stimulatory effect of insulin on lipogenesis in adipocytes, cells were treated with insulin at 10 ng/ml or insulin plus bST at 10, 100 and 1000 ng/ml for 24, 48 or 72 h. Insulin stimulated lipogenesis at all time points (3–6-fold); however, the greatest effects of insulin and bST were observed at 72 h (Table 2). Addition of bST at 10, 100 and 1000 ng/ml to insulin-containing cultures for 72 h decreased lipogenesis by 30 %, 67 % and 62 % respectively (Table 2).

To determine the time course of the ST effect on FAS mRNA levels, adipocytes were treated with insulin (10 ng/ml) or insulin plus bST (100 ng/ml) for 6, 12, 24, 48 or 72 h. Insulin stimulated FAS gene expression at all time points (2–13-fold); however, the greatest effect was observed at 24 h (Figure 2). In the presence of bST, FAS mRNA levels were decreased in a time-dependent manner. The greatest effect of bST was observed at 48 and 72 h; bST decreased FAS mRNA levels by 69 % and 68 % at 48 and 72 h respectively (Table 3). Moreover, after 72 h of culture in the presence of ST, FAS mRNA levels had been decreased to levels observed for cells cultured in the absence of insulin and ST.

Somatotropin decreases FAS mRNA levels by decreasing both the transcription rate of the gene and the stability of mRNA

After establishing that FAS mRNA levels were decreased by ST, we next examined whether this was the result of a decrease in gene transcription and/or a change in mRNA stability. Increased elongation of the FAS nuclear transcripts was observed in cells treated with 10 ng/ml insulin for 48 h (Figure 3). After adjustment of the FAS signal using mouse ST receptor, insulin caused a 4.9 ± 1.4-fold increase in the rate of elongation of FAS nuclear transcripts. Of particular importance is that ST markedly decreased the abundance of the FAS nuclear transcript in cells treated with insulin; transcription rate of the FAS gene was decreased by 82 ± 2 %.

To determine whether ST decreases FAS mRNA levels by increasing the rate of mRNA degradation, studies were conducted to evaluate the effect of insulin and insulin plus bST on the half-life of FAS mRNA in the presence of the transcription inhibitor actinomycin D (5 μg/ml). As shown in Figure 4, ST significantly shortened the half-life of FAS mRNA from 35 to 11 h ($P < 0.01$). Thus, in addition to decreasing transcription, ST also results in an obvious destabilization of FAS mRNA.

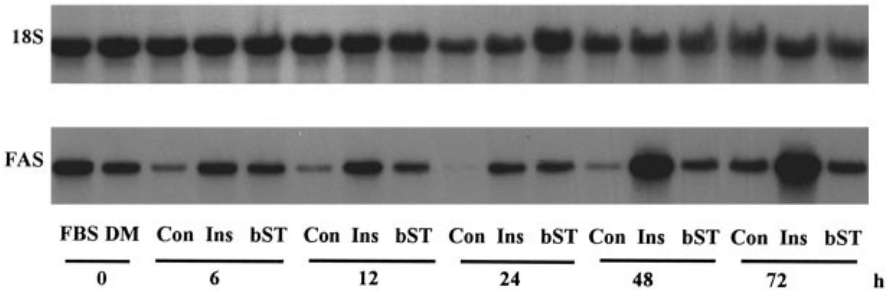

Figure 2 Temporal effects of insulin and bST on FAS mRNA levels in 3T3-F442A adipocytes

Cells were cultured in defined medium (DM), DM plus hydrocortisone (Con) or medium that contained 10 ng/ml of insulin (Ins) or Ins plus bST at 100 ng/ml (bST). Total RNA was harvested at 6, 12, 24, 48 or 72 h. Total RNA was also harvested from adipocytes cultured with media containing FBS (FBS). A representative blot from four independent experiments is shown.

Table 3 Temporal effects of insulin and bST on FAS mRNA levels in 3T3-F442A adipocytes

Cells were cultured in defined medium that contained 10 ng/ml insulin or 10 ng/ml insulin plus 100 ng/ml bST. Total RNA was harvested at 6, 12, 24, 48 or 72 h. FAS mRNA levels were quantified by scanning of autoradiographic signals from Northern blots and normalized with 18 S rRNA to account for differences in loading. Values are means ± S.E.M. for four independent experiments. Statistical comparisons were made within a time period. Means with different superscripts are significantly different ($P < 0.05$).

		FAS mRNA levels (% of control at 6 h)		
Time (h)	Treatment ...	Control	Insulin	Insulin + bST
6		100[a]	231 ± 49[a]	262 ± 60[a]
12		76 ± 18[a]	289 ± 49[a]	254 ± 55[a]
24		28 ± 13[a]	396 ± 122[b]	226 ± 54[ab]
48		39 ± 17[a]	460 ± 175[b]	145 ± 64[a]
72		133 ± 94[a]	538 ± 204[b]	186 ± 72[a]

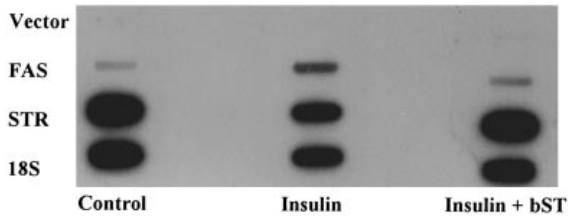

Figure 3 Nuclear run-on transcription of the FAS gene in 3T3-F4422A adipocytes

Nuclei were isolated from adipocytes treated with no hormone (Control), insulin (10 ng/ml) or insulin (10 ng/ml) plus bST (100 ng/ml) for 2 days before the nuclear transcription assay was conducted. A representative blot from three independent experiments is shown.

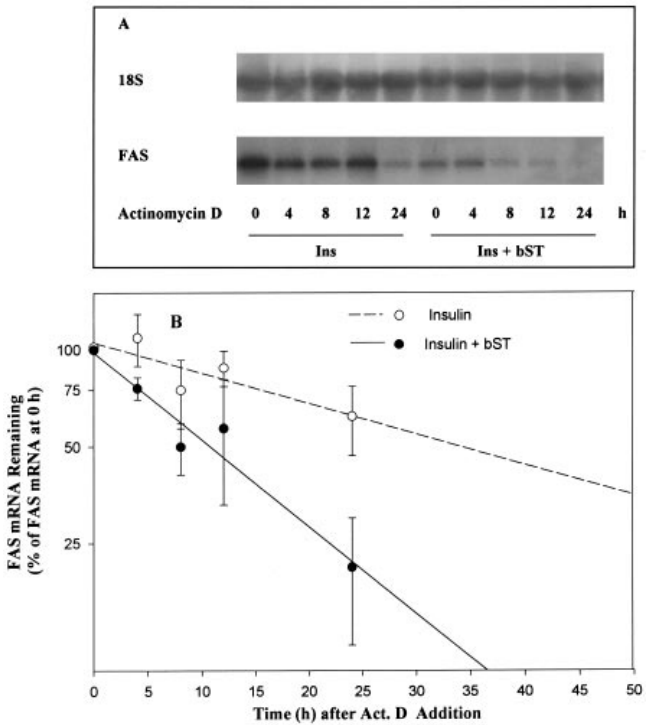

Figure 4 Effect of bST on the stability of FAS mRNA

Adipocytes were cultured with either insulin (10 ng/ml) or insulin (10 ng/ml) plus bST (100 ng/ml) or insulin (10 ng/ml) plus bST (100 ng/ml) for 2 days. Actinomycin D (Act. D) was then added at 5 μg/ml over a 24 h time course. Total RNA was isolated from adipocytes treated with insulin or insulin plus bST at 0, 4, 8, 12 and 24 h after the addition of actinomycin D. (**A**) A representative blot; blots were scanned and normalized with 18 S rRNA to account for differences in loading. (**B**) Quantitative results of five experiments; data are means ± S.E.M. and are plotted relative to the 0 h actinomycin D treatment by using a log scale for the percentage of mRNA remaining.

Somatotropin decreases the stimulatory effect of insulin on the expression of FAS–CAT fusion gene

To corroborate the findings that ST decreased transcription as measured with run-on analysis, we conducted subsequent experiments in which we evaluated the effects of ST on the activity of a FAS–CAT reporter gene that contained the 5′ flanking region (−2195 to +65) of the rat FAS gene. A previous study [19] had demonstrated the presence of an insulin response element (IRE) in the proximal region of the rat FAS promoter (from −71 to −50); however, we elected to study a reporter gene construct that contained an additional 5′ flanking sequence to increase the likelihood that the element(s) that might be important for the ST effect were present. When insulin was added to the culture medium, CAT activity was increased 4.7-fold (Table 4); when cells were cultured with both insulin and bST, CAT activity was decreased by 62 %. Thus, on the basis of two lines of experimental evidence (the run-on transcription results and the FAS–CAT

Table 4 Effect of insulin and bST on expression of FAS–CAT fusion gene (pFAS–CAT5) in stably transfected 3T3-F442A adipocytes

The 3T3-F442A preadipocytes were stably transfected with pFAS–CAT5 containing a portion of the 5′ flanking region of the rat FAS gene (−2195 to +65). The transfected cells were differentiated and treated with no hormone (control), insulin (10 ng/ml) or insulin (10 ng/ml) plus bST (100 ng/ml) for 2 days. The CAT activity data are expressed as a percentage of CAT activity in control cells from three independent experiments ($n = 7$). Values shown are means ± S.E.M. Means with different superscripts are significantly different ($P < 0.05$).

Treatment	CAT activity (% of control)
Control	100 ± 8^{a}
Insulin	465 ± 69^{b}
Insulin + bST	177 ± 29^{a}

data), it is apparent that ST has a major effect on FAS gene transcription.

DISCUSSION

A number of studies have shown that pig ST markedly decreases adipocyte lipogenesis in adipocytes of growing pigs and that this occurs because ST reduces insulin sensitivity (reviewed in [1]). More recently we observed that pST decreases hepatic FAS mRNA levels by 55 % in rats and that this was associated with an 80 % decrease in transcription of the FAS gene [7]. The present study extends these findings to show that ST also decreases transcription of the FAS gene in 3T3-F442A adipocytes. In addition, our evidence clearly indicates that ST also has a significant effect on FAS mRNA turnover. Although 3T3-F442A adipocytes have been widely used to study a variety of mechanisms involved in ST signalling, there have been relatively few studies conducted that have examined the insulin-antagonistic effects of ST [15,20–22], and none of these have assessed the anti-insulin effects of ST on lipogenic enzyme gene transcription. Thus the results of the present study indicate that 3T3-F442A adipocytes are an appropriate cell model to use for investigating the insulin-antagonistic effects of ST.

Little is known about how ST affects the insulin signal pathway(s) to inhibit the stimulatory effects that insulin has on FAS gene expression. Our previous studies have shown that the ST-dependent decrease in insulin sensitivity of pig adipocytes does not seem to be the result of any change in the early events of insulin signalling, such as insulin binding to its receptor on insulin receptor kinase activation [2], suggesting that ST alters insulin signalling event(s) at some point(s) after the insulin receptor. Beyond this, however, little is known about how ST interferes with insulin signal transduction in the adipocyte. Argetsinger et al. [23] have reported that ST stimulates tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1), which is an important molecule for insulin signalling including the rapid recruitment of glucose transporters to the plasma membrane. However, the finding that ST stimulates tyrosine phosphorylation of IRS-1 is difficult to reconcile with the fact that ST has potent insulin-antagonistic effects in adipocytes [1]. One plausible consideration is that phosphorylation of other amino acids in IRS-1 modulates its ability to regulate activation of downstream insulin responses. For example, it has been shown that tumour necrosis factor α, a member of the cytokine family to which ST belongs, can induce IRS-1 to act as an inhibitor of insulin receptor kinase action and subsequent biological effects of insulin [24,25]. An interesting aspect of this observation is that the effects of tumour necrosis factor α seem to be attributable to an increase in serine phosphorylation [24,25]. Whether ST promotes serine phosphorylation of IRS-1 is, to our knowledge, a question that has not been answered. There is evidence that ST can stimulate serine phosphorylation of STAT 1, 3 and 5 [26], which provides indirect evidence to support the idea that the activated ST receptor can phosphorylate proteins on serine residues. However, it remains to be seen whether the ST receptor can phosphorylate serine residues on IRS-1.

With respect to how ST might interfere with insulin signalling at the distal end of the signal pathway (i.e. at the level of FAS gene transcription), we speculate that there are two general mechanisms by which this might occur. The first is that there might be a distinct STRE in the FAS gene that acts as a negative control element. The second consideration is that ST affects the abundance of *trans*-acting factor(s) that interact with the FAS-IRE. In the IRE there is an E-box DNA-binding motif (from −68 to −60) for the basis-helix–loop–helix transcription factors such as upstream stimulatory factors (USFs). Moreover both USF-1 and USF-2 have been shown to bind to this site [27]. Of particular interest is the observation that in rat liver the abundance of USF-1 and the binding of USF-1 to this E-box are regulated by nutritional status [27]; USF-1 was barely detectable in liver nuclear extracts from fasted rat, whereas USF-1 abundance was markedly increased in liver extracts from rats that had been refed after a fast. It is conceivable that USF-1 is responsible for the ST effects because ST treatment, both *in vivo* and *in vitro*, mimics the effects of fasting on FAS enzyme activity, mRNA abundance and gene transcription. Thus it is plausible that ST affects signalling to the IRE by altering the abundance of USF-1 or the binding of USF-1 to the FAS-IRE. However, we recently found that neither the abundance of USF-1 in 3T3-F442A adipocytes nor the binding of USF-1 to FAS-IRE was affected by insulin or ST (D. Yin and T. D. Etherton, unpublished work). Thus USF-1 does not seem to be involved in the regulation of FAS gene transcription by insulin or ST. It remains to be established whether other *trans*-acting factor(s) bind to the FAS-IRE to confer ST regulation of FAS gene transcription. In addition, our evidence indicates that ST decreases FAS mRNA abundance by increasing mRNA degradation. This is the first report that FAS mRNA can be destabilized by ST. A previous study has shown that glucose induces FAS mRNA levels in HepG2 cells predominantly by enhancing FAS mRNA stability [28]. However, little is known about how FAS mRNA degradation is regulated. Thus, given the findings of the present study, it is evident that ST affects both mRNA synthesis and degradation. Because of this, it will be important to elucidate the underlying signal pathways that mediate the effects that ST has on transcription and FAS mRNA stability.

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