Genetics and Molecular Biology, 29, 1, 180-186 (2006)

www.sbg.org.br

Research Article

Effect of growth hormone on fatty acid synthase gene expression in porcine adipose tissue cultures

Andrea A.F.B.V. José[1], Lúcia. E. Alvares[2], Eduardo F. Delgado[1], Luiz L. Coutinho[1] and Dante. P.D. Lanna[1]

[1]*Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Departamento de Zootecnia, Piracicaba, SP, Brazil.*
[2]*Universidade Federal do Paraná, Departamento de Biologia Celular, Curitiba, PR, Brazil.*

Abstract

We describe an efficient *in vitro* assay to test growth hormone effects on mRNA levels and fatty acid synthase (FAS, EC. 2.3.1.85) activity. Swine adipose tissue explants were long-term cultured in medium containing growth hormone and FAS mRNA levels and enzyme activity were measured. We quantified FAS transcripts by competitive reverse transcriptase PCR (RT-PCR) using total RNA from cultured adipose tissue explants and RT-PCR standard-curves were constructed using a cloned 307 bp segment of native FAS cDNA and a shorter fragment from which a 64 bp (competitor, 243 bp) internal sequence had been deleted. A known amount of competitor was added to each PCR as an internal control and μ-*actin* transcripts were also measured to correct for differences in total RNA extraction and reverse transcription efficiency. In cultures with added growth hormone FAS mRNA levels decreased 70% ($p < 0.01$) and FAS enzyme activity decreased 22% ($p < 0.05$). These *in vitro* growth hormone effects were consistent with those observed *in vivo*, showing that *in vitro* adipose tissue culture combined with RT-PCR is a useful and accurate tool for studying growth hormone modulation of adipose tissue metabolism. This technique allowed the diagnosis of lower levels of FAS mRNA in the presence of growth hormone and these low levels were associated with decreased FAS activity in the adipose tissue explants.

Key words: mRNA quantification, enzyme activity, swine, cultures of adipose tissue explants, competitive RT-PCR.

Received: September 24, 2004; Accepted: August 1, 2005.

Introduction

The mechanisms involved in the control of metabolism by growth hormone have been intensively studied due to the magnitude of its effects. According to Bauman (1999), metabolic alterations caused by treatment with growth hormone involve long-term responses involving coordinated changes in the metabolism of many tissues. These changes allow the use of nutrients by certain tissues during specific phases of animal development or different physiological states. For instance, the partitioning of nutrients may allow a larger flow of nutrients to the mammary gland for milk synthesis during lactation. These long-term effects include a reduced response to insulin as measured by whole body glucose uptake *in vivo* as well as a reduced response to insulin stimulation of lipogenesis *in vivo* and *in vitro* (Bauman and Vernon, 1993). In addition, depending on the energy balance, these metabolic changes may include an increase in lipolysis and a decrease in adipose tissue response to the antilipolytic effects of adenosine (Lanna *et al.*, 1995; Lanna and Bauman, 1999).

Send correspondence to Dante. P.D. Lanna. Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Departamento de Zootecnia, Avenida Pádua Dias 11, 13418-900 Piracicaba, SP, Brazil. E-mail: dplanna@esalq.usp.br.

In growing swine 80% of fatty acid synthesis occurs *de novo* using glucose as substrate because only a low proportion of the calories in their normal feed is present as pre-formed fatty acids and about 40% of body glucose usage is destined for fat deposition in adipose tissue (O'Hea and Leville, 1969; Bauman, 1976; Etherton *et al*., 1995).

Administration of porcine recombinant growth hormone (pGH) to growing pigs for 30-77 days at the maximally effective doses (>100 μg kg^{-1} of body weight) increased average daily gain by approximately 10 to 20%, improved productive efficiency 13 to 33%, decreased lipid accretion rates by 70 to 90% and stimulated protein deposition by around 70% (Etherton *et al*., 1995; Etherton and Bauman, 1998; Etherton, 2004). Treatment with pGH also enhanced protein deposition in well-nourished growing pigs (Vann *et al*., 2000) and increased lysine requirements, which is commensurate with increased protein deposition in boars (King *et al*., 2000). Sows treated with growth hor-

mone at some time during, or even late in, gestation showed an increase in progeny size at birth (Rehfeldt *et al*., 2004). The net benefits of pGH administration are the result of re-partition of nutrients from adipose tissue to muscle deposition, and include lower production costs, better product quality and less excretion of nitrogen into the environment. Perhaps the most important effect is increased feed efficiency because this reduces pressure on world feed supplies and the environment (Sillence, 2004).

Emphasis has been given to the study of growth hormone effects on adipocytes, an important target for this hormone in growing and lactating animals. The main mechanism seems to involve changes in tissue responses to homeostatic signals (Bauman and Vernon, 1993) and in the total activity of rate limiting enzymes, including those controlling lipogenesis and lipolysis (Etherton and Bauman, 1998). Among adipocyte enzymes, fatty acid synthase (FAS, EC. 2.3.1.85) and acetyl-coenzyme A carboxylase (ACC, EC. 6.4.1.2) seem to be particularly important since their activities are parallel to changes in rates of fatty acids synthesis (Sinnet-Smith *et al*., 1979). Therefore, the maximum capacity of adipose tissue to synthesize fatty acids *de novo* is related to the total amount and specific activity of lipogenic enzymes which in turn is dependent on the levels of mRNA that codify these enzymes (Clarke, 1993). Growth hormone attenuates the stimulatory effect of insulin on FAS gene transcription and mRNA concentration by increasing mRNA degradation in 3T3-F442A adipocytes (Yin *et al*., 1998; Yin *et al* 2001a) and is also known to decrease FAS activity and mRNA concentration in isolated porcine adipocytes (Louveau and Gondret, 2004). However the mechanism(s) by which growth hormone interferes with the action of insulin on FAS gene transcription has not yet been elucidated (Louveau and Gondret, 2004).

A clear understanding of the molecular mechanisms by which growth hormone regulates lipogenic enzymes in swine adipose tissue requires the development of *in vitro* methodologies to reduce the costs and labor required to test a large number of samples and treatments. Here we show that the use of long-term cultures of adipose tissue explants in association with the competitive reverse transcriptase polymerase chain reaction (RT-PCR) to quantify FAS mRNA provides a valuable system to analyze the effects of growth hormone in small samples of adipose tissue.

Material and Methods

Swine adipose tissue cultures

Seven Landrace x Large White castrated male pigs with an average weight 78 kg were humanly slaughtered and used as a source of adipose tissue. The pigs had been fed a 78% corn and 19% soybean meal diet which had a 3.3% ether extract (percentages on a dry matter basis). Around 7% of metabolizable energy was coming from lipids and animals had free access to feed and water until slaughter. The pigs had been bred at the Department of Animal Science, Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo (ESALQ-USP), Piracicaba, SP, Brazil.

About 20 g of subcutaneous adipose tissue were removed postmortem from the upper leg (ham) of each pig and immediately placed in transport buffer (25 mM HEPES, 0.15 M NaCl, 37 °C, pH 7.4). In the laboratory, 30 mg sections (explants) of the intermediate layer of the subcutaneous adipose tissue were aseptically removed and placed into multi-well plates containing Medium 199 with Earle's salts, l-glutamine, 25 mM HEPES and 25 mM bicarbonate, pH 7.4, (Sigma Chemical, St Louis, MO) supplemented with antibiotics (Lanna *et al*., 1995). A total of 3 explants per triplicate were used for each hormonal treatment for each pig. Control cultures were supplemented with 0.1 μg mL^{-1} of insulin and 10 nM of dexamethasone, with experimental growth hormone cultures receiving a further addition of 0.1 μg mL^{-1} of pGH (Reporcin®, Southern Cross Biotech, Australia). The cultures were incubated in an atmosphere containing 95% air and 5% CO_2 (v/v) at 37 °C for 48 h, after which the explants (~30 mg) from each pig and treatment were collected, grouped into samples and immediately assayed for FAS activity or subjected to total RNA extraction.

Cloning of native and competitor FAS cDNAs

The plasmid pBFAS5 containing a 1.5 kb fragment of cDNA corresponding to the thioesterase domain of the swine FAS gene (Mildner and Clarke, 1991) was kindly donated by Mildner and Clarke (University of Texas, Austin, USA). The cDNA was partially sequenced using the BigDye Terminator Kit (Applied Biosystems, Foster City, CA) on an ABI PRISM 377 automated DNA sequencer. Based on this sequence, the following oligonucleotide primers (Operon Technologies. Inc, Huntsville, AL) were designed: sense (5' CCTACTACATCGAGTGCATC 3') and antisense (5' GGTATCAGCGCCTCCAGCAC 3'). These primers were used for amplification of 307 bp of the cDNA of the FAS gene which we named the native cDNA fragment. A third primer (5' GGTATCAGCGCCTCCAG CACGCCTCGCAGCCGGGGGTCA 3') was designed to delete an internal stretch of the PCR product of the FAS cDNA to produce a 243 bp fragment denominated by us as the competitor fragment. The PCR assay to generate deleted fragments has been described by Kotake *et al*. (1996). The primers for β-*actin* amplification were designed based on the sequence published by McNeel and Mersmann (1999). The β-*actin* primers were: sense 5' CTAGGCAC CAGGGCGTCATC 3'; antisense 5' CTTAGGGTTCAG GGGGGCCT 3'; and antisense deletion 5' CTTAGGG TTCAGGGGGGCCTTAGAAGGTGTGGTGCCAGA 3'. The fragment corresponding to the β-*actin* native cDNA fragment was 227 bp and the β-*actin* competitor cDNA

fragment was 180 bp. The PCR products corresponding to native and competitor cDNAs were cloned in pGEM4Z/ *Escherichia coli* JM109 using the SureClone Ligation Kit (Pharmacia Biotech, Carlsbad, CA). All clones were sequenced using the BigDye Terminator Kit and the ABI PRISM 377 automated DNA sequencer before performing the quantitative RT-PCR assays.

Standard curves for FAS and β-*Actin*

Standard curves for the quantification of FAS transcripts were obtained by co-amplifying 1:1 serial dilutions of the native cDNA (25.6 to 0.1 x 10^{-3} fmol) with a constant amount of the cDNA competitor (10^{-3} fmol), in 100 ng of total RNA. After PCR, amplified products were loaded onto 4.25% denaturing acrylamide gels and submitted to electrophoresis in an Automated Laser Fluorescent DNA Sequencer (A.L.F.™, Pharmacia LKB Biotechnology, Carlsbad, CA). Following electrophoresis, peak areas of native and competitor products were integrated using the Fragment Manager software (Pharmacia LKB Biotechnology, Carlsbad, CA). The logarithmic proportion between the native and competitor areas (log(N/C)) was plotted on the Y axis while the logarithm of the amount of native cDNA added to the reaction (log Ni) was plotted on the X axis of the standard curve. An equation obtained by linear regression of the standard curves was used to determine the initial number of mRNA molecules for FAS in the samples of adipose tissue explants. The standard β-*actin* curve was obtained in a similar way by modifying the concentration of the native and competitor cDNAs to concentrations of 10^{-4} fmol. A detailed description of the methodology used in our competitive RT-PCR assays has been described by Alvares *et al*. (2003).

Quantitative RT-PCR

After 48 h in culture, 3 to 4 explants (~30 mg each) from each pig and treatment were collected, combined to approximately 100 mg, pulverized in liquid nitrogen and submitted to total RNA extraction with Trizol reagent (Life Technologies, Rockville, MD). Samples were dissolved in RNAse-free water and quantified using the mean of duplicate spectrophotometric readings at 260 nm (A260). Purity of total RNA was determined by the A260:A280 ratio. The integrity of each RNA sample was checked by electrophoresis on 1% agarose gels. cDNA samples were prepared from 1 μg of total RNA, using oligo (dT) primer. The synthesis of cDNAs were performed with the Superscript II Preamplification System kit (Life Technologies. Rockville, MD) according to manufacturers protocol.

The competitive PCRs, containing 2 μL cDNA and a known amount of competitor cDNA, were prepared in a final volume of 25 μL. In addition to templates, the reactions contained 1 X PCR buffer (10 mM tris-HCl pH 8.3, 50 mM KCl, 0.01% Triton X-100), 1.5 mM (FAS) or 1.0 mM $MgCl_2$ (μ-*actin*), 200 μM dNTPs, 5 pM of the sense and antisense primers and 1.25 U of *Taq* DNA polymerase (Life Technologies, Rockville, MD). Sense primers were 5'-labeled with fluorescein for subsequent detection of PCR products in the A.L.F. automated DNA sequencer. The PCR parameters consisted of an initial denaturation at 95 °C for 3 min, followed by annealing at 57 °C for 1 min and extension at 74 °C for 1 min, except for the last cycle when extension took 10 min. The same conditions were used in the PCR programs for amplification of the FAS (24 cycles) and the μ-*actin* fragments (34 cycles).

Enzymatic activity assay

The FAS assay was performed according to ingle *et al*. (1973), with some modifications. Samples of cultured explants for each treatment were homogenized in a polytron in 3 mL of 0.15 M KCl, 50 mmol tris-buffer, 1 mmol glutathione and 1 mmol EDTA buffer at 4 °C and pH 7.4. The FAS activity was calculated spectrophotometrically at 340 nm from the rate of transformation of NADPH to NADP in incubations containing substrate, cofactors and homogenated adipose tissue. The concentrations of the reagents were: 40 mm of potassium phosphate buffer plus EDTA (pH 6.8), 0.1 mm malonyl-CoA, 0.1 mm acetyl-CoA, 0.3 mm NADPH and 0.4 mmol of dithiothreitol. Protein concentration in the homogenate was determined by the Biuret method (Gornall *et al*. 1949) using bovine serum albumin as standard and the activities expressed as nmoles min^{-1} mg^{-1} of cytosol protein.

Statistical analysis

Paired data was analyzed using the *t*-test (SAS, 1999). For mRNA concentration, the FAS values were corrected for the concentration of β-*actin* mRNA. The same statistical design was used for analysis of FAS enzymatic activity.

Results and Discussion

Growth hormone effects on FAS Activity

We found that FAS activity decreased ($p < 0.05$) by 22% as a result of adipose tissue explants being cultured for 48 h in the presence of pGH (Table 1). A similar decrease in the enzymatic activity has been observed in cultures of adipose tissue explants (Lanna *et al*., 1994) and also in isolated adipocytes cultured for 48 h (Louveau and Gondret, 2004). However, the magnitude of the reduction in activity was smaller than that observed with swine adipose tissue explants biopsied after 1 to 4 weeks of *in vivo* treatment with pGH, which showed a 40 to 80% reduction in FAS activity (Harris *et al*., 1993; Donkin *et al*., 1996a; Lee *et al*., 2000). Differences from studies *in vitro* and *in vivo* can be reconciled because of the long FAS protein half-life of around 48 h (Dice and Goldberg, 1975). The *in vivo* studies al-

Table 1 - Effect of porcine growth hormone (pGH) on fatty acid synthase (FAS) activity and FAS mRNA concentration in swine adipose tissue cultured for 48 h.

Fatty acid synthase (FAS)	Control	Experimental	Standard deviation for paired observations	p-values
	Insulin 0.1 μg mL^{-1} + 10nM dexamethasone	As for control + 0.1μg mL^{-1} pGH		
FAS activity[1]	46.4	35.8	9.07	0.032
FAS mRNA[2]	8.7	3.7	0.64	0.17
FAS mRNA[3]	6.1	1.8	0.22	0.003

[1]nmoles of substrate (NADPH) transformed to NADP per min per mg of cytosol protein. [2]Times 10^{-4} femtomoles. [3]Transcripts normalized for β-*actin*.

lowed sufficient time for bST to reduce not only mRNA expression, but to reduce FAS protein concentrations.

Native and competitive FAS cDNA standard curves

The standard curve obtained for FAS mRNA is shown in Figure 1B. As the intensity of native FAS cDNA products decreased, there was, as expected, a gradual increase in the intensity of competitor products (Figure 1A). Bands showing approximately the same signal intensity for the native (3447.1 pixels) and competitor (3459.8 pixels) products were observed at the 3.2 fmols dilution, indicating that the competitor had higher amplification efficiency than the native cDNA. The higher efficiency of the competitor cDNA amplification is reflected in the negative value for the intercept (Alvares *et al*., 2003).

Effect of growth hormone on FAS gene expression

The amount of FAS mRNA in the adipose tissue cultures after 48 h treatment with pGH was calculated both with and without a correction for the β-*actin* housekeeping gene, the uncorrected means showing a non-significant 57% decrease while the β-*actin* corrected values showed a significant decrease of 70% ($p < 0.05$; Table 1). A decrease in FAS mRNA has also been observed when animals were treated *in vivo* with pGH (Harris *et al*., 1993; Mildner and Clarke, 1991; Donkin *et al*., 1996a; 1996b; Yin *et al*., 1998), suggesting that growth hormone causes decreased *FAS* gene expression. Moreover, Yin and co-workers (1998) observed a reduction in the stability of FAS mRNA, whose half-life of 35 h decreased to 11 h when mRNA degradation increased.

Such changes in gene expression support the observed decrease in the capacity of adipose tissue to synthesize lipids as measured by the incorporation of labeled glucose in live pigs (Dunshea *et al*., 1992b; Harris *et al*., 1993; Lanna *et al*., 1994; Donkin *et al*., 1996a; Yin *et al*., 1998) and labeled acetate in ruminants (Ingle *et al*., 1973; Vernon and Finley, 1988; Borland *et al*., 1994; Lanna *et al*., 1995; Lanna and Bauman, 1999). Studies with long-term *in vivo* growth hormone treatment followed by biopsies and evaluation of adipose metabolism *in vitro* by Harris *et al*. (1993) demonstrated that pGH dramatically reduces lipogenesis rates. The work by Harris also demonstrated a sharp decrease in the activity of the enzymes responsible for *de novo* fatty acid synthesis, particularly FAS. This is consistent with the FAS concentration having a decisive role in the maximum capacity of adipose tissue to synthesize fatty acids *de novo* (Clarke, 1993). Along with the changes in FAS activity, the amount of acetyl-CoA carboxylase (ACC) is also known to be reduced in both ruminant and non-ruminant animals treated with growth hormone (Vernon and Finley 1988; Magri *et al*., 1990; Vernon *et al*., 1991; Harris *et al*., 1993; Lanna *et al*., 1995; Lanna and Bauman, 1999).

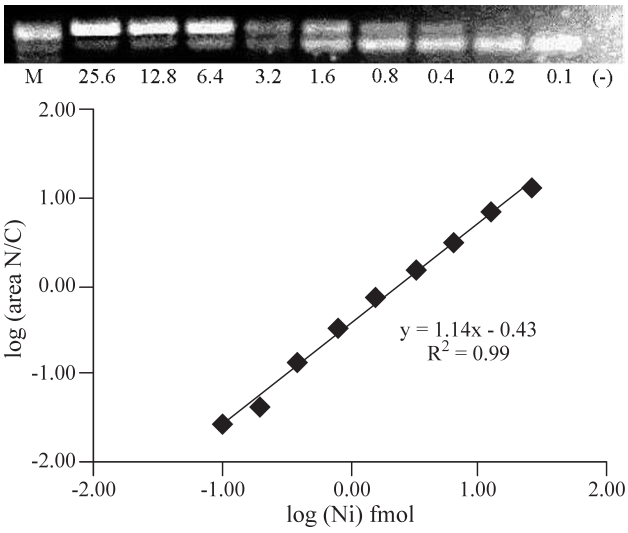

Figure 1 - Fatty acid synthase (FAS) mRNA standard curve. A = Electrophoresis on 3% agarose gel of the FAS standard curve at 10^{-3} femtomoles (fmol). B = Standard curve for FAS mRNA concentration at 10^{-3} fmol. The $\log_{10}$ of the ratio of native to competitor PCR product was plotted against the $\log_{10}$ of the initial native fragment added to the competitive reverse transcriptase PCR (RT-PCR). M = molecular weight marker.

Vernon & Finley (1988) and Vernon *et al*. (1991) observed that growth hormone inhibited the increase in lipogenic rates and ACC activity caused by insulin and dexamethasone and that this effect was abolished by actinomycin D. It appears that growth hormone inhibits the synthesis of key proteins in this process, including factors involved in the transduction of the signals controlling lipogenesis. The growth hormone effects observed by Vernon *et al*. (1991) occurred within 4-8 h, however, due to the long half-lives of FAS and ACC, growth hormone effects during shorter periods should involve components of the activation and signaling pathways of the enzymes, while in the long-term (over 48 h) treatment with growth hormone

would affect the amount of enzymes. Given the half-life of FAS mRNA, a decrease in FAS mRNA should occur earlier than a decrease in the amount of enzyme and total enzymatic activity. Ingle *et al*. (1973) observed that in sheep given access to feed after a fasting period there was a strong correlation ($r = 0.95$) between ACC activity and lipogenesis as measured *in vitro* by the incorporation of labeled acetate into lipids. The synthesis of fatty acids was reduced by 91% after 60 h and 95% after 112 h of feed deprivation although FAS and ACC activities were reduced by only 40% after 96 h of fasting. The slower reductions in lipogenic enzyme activities than in lipid synthesis after feed deprivation seem to be consistent with those observed with growth hormone.

Using the quantitative competitive reverse transcriptase (QC- RT- PCR) described in this study we observed a reduction in the concentration of FAS mRNA in adipose tissue from swine submitted to 48 h fasting, *i.e.* from 10^{-3} fmol g^{-1} in *ad libitum* controls to 10^{-4} fmol g^{-1} for fasted animals (José *et al*., 1999). In that experiment after fasting for 48 h pigs were given access to feed for 24 h and displayed FAS mRNA concentrations similar to controls fed *ad libitum* throughout the period. In these fasting/refeeding studies the QC-RT-PCR technique detected small difference between FAS mRNA levels and demonstrated that QC-RT-PCR may be applied to the evaluation of a variety of diet and hormonal treatments.

Regulation of gene expression of the key enzymes of lipid synthesis is also controlled by several factors including animal age, nutritional state, diet composition, proportion of carbohydrates and fats, profile and degree of saturation of the fatty acids as well as hormonal effects. Girard *et al*. (1997) designed a series of studies demonstrating the role of FAS in the regulation of fat synthesis in the course of the development of mice fed with diets of different composition. They observed, together with Towle *et al*. (1997), that low fat diets with high amounts of simple carbohydrates can induce a group of key enzymes (particularly FAS) involved in lipogenesis in the liver of mammals. This regulatory system seems to be partly effected by the action of intermediate metabolites that activate transcription factors. This activation of transcription factors that lead to changes in the amounts of the mRNA of those key enzymes seems to involve both glucose-6-phosphate and elements of the insulin cascade.

The precise mechanisms involved in the insulin antilipolytic effect are still unclear, but include a reduction in the intracellular concentration of cAMP, stimulation of low Km phosphodiesterase and phosphatases capable of dephosphorylation and inactivation of hormone sensitive lipase. Yin *et al*. (1998) observed an increase of 2 to 13 fold in FAS mRNA concentration and 3 to 7 fold in rates of lipid synthesis in cells treated with insulin and found that growth hormone reduced the stimulatory effects of insulin on FAS mRNA concentration by 40 to 70% and lipogenesis by 20 to 60%.

In swine, the most important effect of growth hormone in adipose tissue results from changes in lipogenesis, with lipolysis rates being relatively unaffected (Etherton and Bauman, 1998 and Dunshea *et al*., 1992a). Dunshea *et al*. (1992a) administered radioactively labeled glucose and fatty acids to growing pigs treated with pGH and found that in pigs with a positive energy balance decreases in lipid deposition were the result of decreased lipid synthesis without any changes in rates of fatty acid mobilization from body reserves (Dunshea *et al*., 1992a).

In our adipose tissue cultures with insulin and dexamethasone, addition of pGH caused a decrease in FAS gene expression, expressed as a 22% reduction in total enzymatic activity (significant at $p < 0.05$) and a 70% reduction in FAS mRNA (significant at $p < 0.01$). Under these *in vitro* conditions the addition of pGH to the culture medium did not affect the concentration of μ-*actin* mRNA ($p = 0.74$).

In our experiments there was no insulin-free control, and thus it may be argued that pGH only inhibited insulin stimulation of FAS gene transcription. It is indeed possible that the pGH might only act indirectly through modulation of the insulin-signaling cascade. The mechanism by which growth hormone interferes with the action of insulin on FAS gene transcription has not yet been totally elucidated (Louveau and Gondret, 2004). Magri *et al*. (1990) observed that a decrease in insulin sensibility caused by growth hormone in swine adipose tissue was neither associated with changes in the number of insulin receptors nor in tyrosine kinases activity. Our laboratory has demonstrated a decrease in PI3 kinase associated with both IRS-1 (insulin receptor substrate) and IRS-3 in adipose tissue (Castro *et al*., 2004) while others have demonstrated the same for IRS-1 and IRS-2 in muscle (Thirone *et al*., 1997, 1998). According to Donkin *et al*. (1996b) two mechanisms can exist by which pGH can antagonize insulin effects on *FAS* transcription. One mechanism could involve interference with the signaling cascade reducing the trans-activation factors which interact with key *cis* elements of the insulin response (IRE) factors which are positive regulators of *FAS* transcription. The other possible mechanism is a growth hormone response element, or somatotrophic response element (STRE), in the *FAS* gene that acts as a negative control. Such a response element has indeed been demonstrated in the gene. Yin *et al*. (2001b) suggested that the somatotrophic response element may be located within the first 112 bp of the *FAS* promoter. One possible candidate is the sterol regulatory element binding protein (SREBP)-1c, a member of the helix-loop-leucine zipper family of transcription factors, which has been reported to play a pivotal role in mediating the effects of insulin in liver (Foufelle and Ferré, 2002). Frick *et al* (2002) observed that when growth hormone was administered to rats for 7 days there was increased expression of FAS and SREBP-1c in the livers of the rats. Insulin and growth hormone had no additive effects on these genes, instead insulin blunted the effect of

growth hormone on SREBP-1c mRNA. In contrast to liver, adipose tissue expression of FAS and SREBP-1c mRNA was not influenced by growth hormone. Frick *et al*. (2002) concluded that the increased hepatic expression of lipogenic enzymes after growth hormone treatment may be explained by increased expression of SREBP-1c and insulin does not mediate the effects of growth hormone but inhibits the stimulatory effect of growth hormone on hepatic SREBP-1c expression and triglyceride secretion rate. The same observation was made by Louveau and Gondret (2004) who treated isolated porcine adipocytes with growth hormone and insulin and found that the regulation of lipogenesis appears not to involve changes in SREBP-1 mRNA levels. Glucose incorporation and FAS activity were increased by insulin in a dose-dependent manner and addition of pGH inhibited insulin-stimulated FAS activity. Such inhibition was strongly paralleled by variations in FAS mRNA, after 2 days of culture. These data are consistent with our results where pGH decreased FAS activity and FAS mRNA concentration after 2 days of culture even though the culture systems and evaluation methods were different to those of Louveau and Gondret (2004).

Besides the work developed with primary adipose tissue culture, including the system described by us in this paper, studies with cell lineages such as 3T3-F442A have been used for observations of the effects of several treatments affecting lipogenesis including growth hormone (Yin *et al*., 1998, Yin *et al*., 2001a,b). Data from these studies suggest that cell lineages are an appropriate model for the investigation of the antagonistic effects of growth hormone on insulin. The magnitude of the reductions in the concentration of FAS mRNA observed by Yin *et al.* in the studies cited above was close to that observed by us.

Despite the consistency of the data from cell lineages and primary cultures of adipocytes, cell lineages differ metabolically from normal cells in several ways, including the fact that they are immortal. Therefore even though cell lineage data agree with other reports cell linage data should still be regarded with caution.

Conclusions

Using competitive reverse transcriptase PCR (RT-PCR) and long-term cultures of adipose tissue explants, the growth hormone effects on gene expression of the lipogenic enzyme FAS can be evaluated in a defined medium. Our results were very similar to those from other *in vitro* and *in vivo* work in that we found that chronic treatment with porcine growth hormone (pGH) decreased fatty acid synthase (FAS) gene expression as measured both by mRNA content and total enzymatic activity of swine adipose tissue.

Acknowledgments

We are grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the scholarship and grant that supported this work and to CNPq for the fellowships to Dante P. D. Lanna and Luiz L. Coutinho. We also would like to thank Southern Cross Biotech for donating pGH. We also thank Dr. Ana M. Mildner, Dr. Steven D. Clarke and Dr. Margarita Teran-Garcia who kindly donated the clone containing the swine FAS insert and Dr. Valdomiro S. Myiada and the employees of the Swine Unit of ESALQ-USP.

References

Alvares LE, Mantoani A, Corrente JE and Coutinho LL (2003) Standard-curve competitive RT-PCR quantification of myogenic regulatory factors in chicken embryos. Braz J Med Biol Res 36:1629-1641.

Bauman DE (1976) Intermediary metabolism of adipose tissue. Fed Proc 35:2308-2313.

Bauman DE (1999) Bovine somatotropin and lactation: From basic science to commercial application. Domest Anim Endocrinol 17:101-116.

Bauman DE and Vernon RG (1993) Effects of exogenous bovine somatotropin on lactation. Annu Rev Nutr 13:437-461.

Borland CA, Barber MC, Travers MT and Vernon RG (1994) Growth hormone inhibition of lipogenesis in sheep adipose tissue: Requirement for gene transcription and polyamines. J Endocrinol 142:235-243.

Castro FCP, Delgado EF, Bezerra RMN and Lanna DPD (2004) Effects of growth hormone on insulin signal transduction in rat adipose tissue maintained *in vitro*. Endocr Res 30:225-238.

Clarke SD (1993) Regulation of fatty acid synthase gene expression: An approach for reducing fat accumulation. J Anim Sci 71:1957-1965.

Dice JF and Goldberg AL (1975) Statistical analysis of the relationship between degradative rates and molecular weights of proteins. Arch Biochem Biophys 170, 213-219.

Donkin SS, Chiu PY, Yin D, Louveau I, Swencki B, Vockroth J, Evock-Clover CM, Peters JL and Etherton TD (1996a) Porcine somatotropin differentially down-regulates expression of GLUT4 and Fatty Acid Synthase genes in pig adipose tissue. J Nutr 126:2568-2577.

Donkin SS, Mcnall AD, Swencki BS, Peters JL and Etherton TD (1996b) The growth hormone – Dependent decrease in hepatic fatty acid synthase mRNA is the result of decrease in gene transcription. J Mol Endocrinol 16:151-158.

Dunshea FR, Harris DM, Bauman DE, Boyd RD and Bell AW (1992a) Effect of somatotropin on nonesterified fatty acid and glycerol metabolism in growing pigs. J Anim Sci 70:132-140.

Dunshea FR, Harris DM, Bauman DE, Boyd RD and Bell AW (1992b) Effect of porcine somatotropin on *in vivo* glucose kinetics and lipogenesis in growing pigs. J Anim Sci 70:141-151.

Etherton TD (2004) Somatotropic function: The somatomedim hypothesis revisited. J Anim Sci 82(E. Suppl.):E239-E244.

Etherton TD and Bauman DE (1998) Biology of Somatotropin in Growth and lactation of domestic animals. Physiol Rev 78:745-761.

Etherton TD, Donkin SS and Bauman DE (1995) Mechanisms by which porcine somatotropin (pST) decreases adipose tissue growth in growing pigs. In: Smith SB, Smith DR and Champaign Il. The Biology of Fat in Meat Animals. Am Soc Anim Sci, Ithaca, pp 53-69.

Frick F, Lindén D, Améen C, Edén S, Mode A and Oscarsson J (2002) Interaction between growth hormone and insulin in the regulation of lipoprotein metabolism in the rat. Am J Physiol Endocrinol Metab 283:E10234-E1031.

Foufelle F and Ferré P (2002) New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: A role for the transcriptional factor SREBP-1c. Biochem J 366:377-391.

Girard J, Chatelain F, Boillot J, Prip-Buus C, Thumelin S, Pégorier JP, Foufelle F and Férre P (1997) Nutrient regulation of gene expression. J Anim Sci 75:46-57.

Gornall AG, Bradawill CT and David MM (1949) Determination of serum protein by means of biuret reagent. J Biol Chem 177:751-766.

Harris DM, Dunshea FR, Bauman DE, Boyd RD, Wang SY, Johnson PA and Clarke SD (1993) Effect of *in vivo* somatotropin treatment of growing pigs on adipose tissue lipogenesis. J Anim Sci 71:3293-3300.

Ingle DL, Bauman DE, Mellenberger RW and Johnson DE (1973) Lipogenesis in the ruminant: Effect of fasting and refeeding on fatty acid synthesis and enzymatic activity of sheep adipose tissue. J Nutr 103:1479-1488.

José AAFBV, Alvares LE, Coutinho LL and Lanna DPD (1999) Padronização da técnica de RT-PCR quantitativo para avaliação da expressão gênica da FAS em suínos. Gen Mol Biol 22:639 (abstract).

King RH, Campbell RG, Smits RJ, Morley WC, Ronnfeldt K, Butler K and Dunshea FR (2000) Interrelationships between dietary lysine, sex, and porcine somatotropin administration on growth performance and protein deposition in pigs between 80 and 120 kg of live weight. J Anim Sci 78:2639-2651.

Kotake S, Jr-Schumacher HR and Wilder RL (1996) A simple nested RT-PCR method for quantification of the relative amounts of multiple cytokine mRNAs in small tissue samples. J Immunol Methods 199:193-203.

Lanna DPD, Dwyer DA and Bauman DE (1994) Somatotropin and the control of lipogenesis by insulin and adenosine in pig adipose tissue. J Anim Sci 72:161.

Lanna DPD, Houseknecht KL, Harris DM and Bauman DE (1995) Effects of somatotropin treatment on lipogenesis, lipolysis and related cellular mechanisms in adipose tissue of lactating cows. J Dairy Sci 78:1703-1712.

Lanna DPD and Bauman DE (1999) Effect of somatotropin, insulin, and glucocorticoid on lipolysis in chronic cultures of adipose tissue from lactating cows. J Dairy Sci 82:60-68.

Lee KC, Azain MJ, Hausman DB and Ramsay TG (2000) Somatotropin and adipose tissue metabolism: Substrate and temporal effects. J Anim Sci 78:1236-1246.

Louveau I and Gondret F (2004) growth hormone and insulin affect fatty acid synthase activity in isolated porcine adipocytes in culture without any modifications of sterol regulatory element binding protein-1 expression. J Endocrinol 181:271-280.

Magri KA, Adamo M, Leroith D and Etherton TD (1990) The inhibition of insulin action and glucose metabolism by porcine growth hormone in porcine adipocytes is not the result of any decrease in insulin binding or insulin receptor kinase activity. Biochem J 266:107-113.

McNeel RL and Mersmann HJ (1999) Distribution and quantification of beta1-, beta2-, and beta3- adrenergic receptor subtype transcripts in porcine tissues. J Anim Sci 77:611-621.

Mildner AM and Clarke SD (1991) Porcine fatty acid synthase: Cloning of a complementary DNA, tissue distribution of its mRNA and suppression of expression by somatotropin and dietary protein. J Nutr 121:900-907.

O'Hea EK and Leville GA (1969) Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. J Nutr 99:338-344.

Rehfeldt C, Nissen PM, Kuhn G, Vestergaard M, Ender K and Oksbjerg N (2004) Effects of maternal nutrition and porcine growth hormone (pGH) treatment during gestation on endocrine and metabolic factors in sows, fetuses and pigs, skeletal muscle development, and postnatal growth. Domest Anim Endocrinol 27:267-285.

SAS Institute, Inc. (1999) SAS/SAT User's Guide. SAS Institute Inc., Cary, NC.

Sillence MN (2004) Technologies for the control of fat and lean deposition in livestock. Vet J 167:242-257.

Sinnet-Smith PA, Vernon RG and Mayer RJ (1979) Fatty acid synthesis and the activities of fatty acid synthesizing in rat adipose tissue during pregnancy and lactation. Biochem Soc Trans 7:388-389.

Thirone ACP, Carvalho CR, Brenelli SL, Velloso LA and Saad MJ (1997) Effect of chronic growth hormone treatment on insulin signal transduction in rat tissues. Mol Cell Endocrinol 130:33-42.

Thirone ACP, Paez-Espinosa EV, Carvalho CRO and Saad MJ (1998) Regulation of insulin stimulated tyrosine phosphorylation of Shc and IRS-1 in the muscle of rats: Effect of growth hormone and epinephrine. FEBS Lett 421:191-196.

Towle HC, Kaytor EN and Shih H (1997) Regulation of the expression of lipogenic enzyme genes by carbohydrate. Annu Rev Nutr 17:405-433.

Vann RC, Nguyen HV, Reeds PJ, Burrin DG, Fiorotto ML, Steele NC, Deaver DR and Davis TA (2000) Somatotropin increases protein balance by lowering body protein degradation in fed, growing pigs. Am J Physiol Endocrinol Metab 278:E477-E483.

Vernon RG, Barber MC and Finley E (1991) Modulation of the activity of acetyl-CoA carboxilase and other lipogenic enzymes by growth hormone, insulin and dexamethasone in sheep adipose tissue and relationship to adaptations to lactation in sheep adipose tissue and relationship to adaptations to lactation. Biochem J 274:543-548.

Vernon RG and Finley E (1988) Roles of insulin and growth hormone in the adaptations of fatty acid synthesis in white adipose tissue during the lactation cycle in sheep. Biochem J 256:873-878.

Yin D, Clarke SD, Peters JL and Etherton TD (1998) 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. Biochem J 331:815-820.

Yin D, Griffin MJ and Etherton TD (2001a) Analysis of the signal pathways involved in the regulation of fatty acid synthase gene expression by insulin and somatotropin. J Anim Sci 79:1194-1200.

Yin D, Griffin MJ and Etherton TD (2001b) Transcriptional regulation of fatty acid synthase gene by somatotropin in 3T3-F442A adipocytes. J Anim Sci 79:2336-2345.

Associate Editor: Klaus Hartfelder