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Chronic Effects of Somatotropin Treatment *in Vivo* and *in Vitro* on Lipogenic Activity of Goat Adipose Tissue in a Glucose-Free Buffer During Acute Incubation

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Summary

Young castrated male goats (n = 8) were used to investigate the effect of long-term treatment with recombinant methionyl bovine somatotropin in a sustained release vehicle (bST; 100 mg at seven-day intervals in a 147-day experiment) and chronic culture (24 h) of omental adipose tissue in the presence of various hormones on lipogenic responses to catecholamines during acute incubation (2 h) in a sodium acetate supplemented glucose-free buffer. The rate of fatty acid synthesis in freshly-prepared adipose explants was low and did not differ from those cultured in the absence of hormones for 24 h. Hormonal combination of insulin (17 $nmol.l^{-1}$) plus cortisol (138 $nmol.l^{-1}$) or insulin plus recombinant enterokinase linker bST (4.5 $nmol.l^{-1}$) increased lipogenesis ($P<0.05$). Further addition of bST or cortisol decreased lipogenesis significantly ($P<0.05$) in the controls but not significantly in bST-treated animals. Cultured explants from either control or bST-treated animals showed significant inhibition of lipogenesis by both norepinephrine (10 $\mu mol.l^{-1}$) and isoprenaline (10 $\mu mol.l^{-1}$). BST treatment *in vivo* did not increase the responsiveness of cultured explants to norepinephrine *in vitro,* however, the responsiveness to isoprenaline(inhibition of lipogenesis) was greater in bST-treated animals than in the controls.

Key words
Goat • Adipose tissue • Somatotropin • Cortisol • Lipogenesis

Introduction

Somatotropin (ST) increases the productive efficiency of farm animals by coordination of the metabolism in many tissues resulting in a greater proportion of depot and absorbed nutrients being partitioned to support lactation or growth (Bauman and Vernon 1993, Slaba *et al.* 1994). The adipose tissue plays a central role in the metabolic adaptations of energy metabolism that occur in growing and lactating animals.

ST is an important regulator of adipose tissue metabolism. In non-ruminants, the effects of ST on

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metabolism can be broadly grouped as either insulin-like (early transient actions) or anti-insulin actions (delayed actions with a lag period of 2-4 h). The insulin-like effects of ST involve enhanced glucose and amino acid utilization and antilipolysis, while the anti-insulin actions include inhibition of glucose utilization and related metabolic pathways and enhanced lipolysis. It has been demonstrated that the insulin-like action of ST is independent of the hormone effect on carbohydrate metabolism, since antilipolysis occurs in the absence of glucose. ST alone has a minimal effect on both lipolysis and lipogenesis unless a glucocorticoid is added (Davidson 1987, Etherton *et al.* 1987, Vernon and Finley 1988, Škarda 1998). However, the mechanism of ST effects on adipose tissue metabolism is far from being clear. It is possible that the main effect of ST is to potentiate the tissue response to other lipolytic agents (e.g. catecholamines) and to antagonize insulin action on glucose metabolism and fatty acid synthesis.

The pathways of fatty acid synthesis in ruminant adipose tissue are markedly different from non-ruminant tissues. Ruminants utilize acetate but not glucose as a carbon source for *de novo* fatty acid synthesis. The inability of ruminant adipose tissue to utilize glucose as a substrate for fatty acid synthesis relates to the extremely low activities of two key enzymes, ATP-citrate lyase and NADP-malate dehydrogenase (Hanson and Ballard 1967, Škarda and Bartoš 1969). Reducing equivalents for fatty acid synthesis are generated in the pentose cycle and isocitrate cycle (Bauman 1976). The advantage of the isocitrate cycle is that acetate can be utilized to generate NADPH. Thus, the ability of ruminant adipose tissue to utilize acetate for the synthesis of both a carbon skeleton of fatty acids and essential NADPH may be used to study the effect of hormonal priming *in vivo* or *in vitro* on acute lipogenic responses in the absence of glucose. In the present experiments on young castrated male goats, long-term bovine ST (bST) treatment *in vivo* was coupled to chronic (24 h) *in vitro* cultures of adipose explants in the presence of insulin (I), cortisol (H) and bST alone or in different combinations to study the ability of these hormones to modulate the lipogenic response to catecholamines in acute (2 h) incubations in a glucose-free buffer.

Methods

Animals and general procedures

The experiments were carried out on 8 young castrated male goats (Czech white breed). Animals were castrated at the age of 2-3 weeks and used for experiments between 2-8 months of age. Animals were arranged in pairs (placebo-treated and bST-treated) according to age (the pairs did not differ by more than one day) and body weight (pairs did not differ by more than 2 kg of body weight) (Škarda 1999). At the age of 2-3 months, placebo-treated animals received a subcutaneous injection of olive oil and bST-treated animals received 100 mg of recombinant methionyl bST (Sometribove; Monsanto Agricultural Co., St. Louis, MO, U.S.A.) in a lipid-based sustained release vehicle at seven-day intervals in a 147-day experiment. After 147 days of treatment all animals were exsanguinated following stunning with a captive bolt. The greater omentum was aseptically removed and placed immediately into sterile phosphate buffered saline at about 38 °C. The omental adipose tissue was cut into explants of about 5-10 mg.

Source of chemicals

The chemicals (analytical grade and hormone solubilization) were described earlier (Škarda *et al.* 1992, Slaba *et al.* 1994, Škarda 1998).

Tissue cultures

Adipose tissue explants intended for culture were transferred into plastic Petri dishes and cultured at 38 °C using modified Waymouth's medium (Škarda 1999) in the presence or absence of insulin (I) (17 $nmol.l^{-1}$), cortisol (H) (138 $nmol.l^{-1}$), and enterokinase linker recombinant bST (Eli Lilly, Indianapolis, IN, U.S.A.; 4.5 $nmol.l^{-1}$).

Tissue incubation

Fresh or cultured adipose tissue explants were transferred to 30 ml polyethylene flasks containing 3 ml (38 °C) of modified sodium acetate (4 $mmol.l^{-1}$) supplemented glucose-free Krebs-Henseleit bicarbonate buffer (Škarda 1999). To measure the responsiveness to catecholamines, the lipogenic activities of adipose explants were assessed in the presence and absence of either norepinephrine bitartarate (NE; 10 $\mu mol.l^{-1}$) or isoprenaline hydrochloride (ISO; 10 $\mu mol.l^{-1}$).

Adipose tissue fatty acid synthesis

Fatty acid synthesis was assessed by measuring the incorporation of sodium (1-^{14}C)acetate (20 $kBq.ml^{-1}$) into total lipids of adipose explants over a 2-h period of incubation at 38 °C (Škarda 1998b). The synthetic activities of adipose explants were expressed in nanomoles of acetate incorporated per mg protein per hour.

Table 1. Effects of long-term treatment with bST *in vivo* and chronic (24 h) culture in the presence of various hormones on the rate of lipogenesis in a glucose-free buffer during acute incubation (2 h) in the presence and absence of catecholamines in adipose explants from young castrated male goats.

Culture variables	Acetate incorporation into fatty acids ($nmol.mg^{-1}.h^{-1}$) — Incubation variables: No catecholamines	NE	ISO
Placebo-treated animals			
Before culture			
No hormones	1.84^{Ca}	1.21^{Ba}	1.42^{Ca}
After culture			
No hormones	5.73^{BCa}	1.50^{Bb}	2.39^{BCc}
Insulin (I)	12.40^{ABa}	2.74^{ABb}	3.77^{ABc}
bST	4.73^{BCa}	1.32^{Bb}	2.15^{Bcb}
Cortisol (H)	2.40^{Ca}	1.24^{Ba}	1.35^{Ca}
I + H	17.08^{Aa}	4.50^{Ab}	4.90^{Ab}
I + bST	14.85^{Aa}	3.39^{ABb}	4.16^{ABb}
I + H + bST	6.90^{BCa}	3.10^{ABa}	3.78^{ABa}
Overall mean	9.16^{a}	2.54^{b}	3.21^{c}
bST-treated animals			
Before culture			
No hormones	1.70^{Ba}	0.52^{Bb}	0.99^{Ba}
After culture			
No hormones	2.78^{ABa}	1.12^{Bb}	1.42^{ABab}
Insulin (I)	5.61^{ABa}	2.65^{Bb}	2.82^{ABb}
bST	2.23^{Ba}	1.13^{Bb}	1.33^{ABb}
Cortisol (H)	1.37^{Ba}	0.87^{Ba}	0.65^{Ba}
I + H	10.44^{Aa}	5.93^{Aa}	3.57^{Ab}
I + bST	5.07^{ABa}	2.68^{Bb}	2.81^{ABb}
I + H + bST	3.48^{ABa}	2.44^{Bb}	1.89^{ABb}
Overall mean	4.43^{a}	2.40^{b}	2.07^{b}
Pooled SEM_1	2.71	0.84	0.78
Pooled SEM_2	0.96	0.30	0.28
Effect of bST treatment	P-0.0036	NS	P-0.0097

Omental adipose explants of four control (placebo-treated) and four bST-treated animals were exposed before and after culture to (1-^{14}C)acetate (20 kBq.ml^{-1}) for a 2-h period of incubation in the presence or absence of norepinephrine (NE; 10 μmol.l^{-1}) or isoprenaline hydrochloride (ISO; 10 μmol.l^{-1}) in Krebs-Henseleit buffer supplemented with sodium acetate (4 mmol.l^{-1}). Cultured explants were maintained in tissue culture for 24 h in a modified Waymouth's medium (containing 3.5 mmol.l^{-1}glucose) in the absence of hormones or in the presence of insulin (17 nmol.l^{-1}), recombinant bST (Somidobove; 4.5 nmol.l^{-1}) and cortisol (138 nmol.l^{-1}) alone or in different combinations. All values are the means of four experiments, each performed in triplicate, and expressed per mg protein. For each column of results, pooled S.E.M.$_1$ (standard error LS mean for paired values) is for comparison of mean values from animals within the same treatment (placebo- or bST-treated); pooled S.E.M.$_2$ (standard error LS mean for unpaired values) is compared with mean values of placebo-treated and bST-treated animals. Values within a column which do not have the same upper case superscript (A, B, C) differ significantly ($P<0.05$). The effects of NE and ISO in uncultured explants and in explants cultured in the presence of different hormones were compared by paired t-test. Values within a row which do not have the same lower case superscript (a, b, c), differ significantly ($P<0.05$).

Protein determination

The protein content of adipose explants was determined after lipid extraction according to Lowry *et al.* (1951) with bovine serum albumin as the standard.

Statistical methods

The results are expressed as means ± S.E.M. Differences among groups of uncultured and cultured explants were analyzed by repeated measure analysis of variance, factors comprising hormones in culture, catecholamines in incubation and their interaction. All calculations were carried out by the GLM Procedure (SAS 1989).

Results

The rate of fatty acid synthesis in freshly-prepared adipose explants incubated for 2 h in a modified glucose-free Krebs-Henseleit buffer was low and non-significantly different from those cultured for 24 h in a modified Waymouth's tissue culture medium without hormones. Addition of hormones into the culture medium changed subsequent lipogenesis in a glucose-free buffer in tissues from both groups of animals in a similar manner. However, overall mean values of lipogenesis in cultured explants incubated in the absence of catecholamines were consistently lower (P=0.0036) in bST-treated animals than in the controls (4.43±0.96, 9.16±0.96 $nmol.mg^{-1}.h^{-1}$, Table 1).

Fatty acid synthesis in explants cultured in the absence of hormones was taken as a basic measure of the lipogenic response to different hormones added to the culture medium. Addition of insulin stimulated the rate of lipogenesis in tissues of control and bST-treated animals by 116.4 % and 101.8 %, respectively. However, the effects of insulin were not significant due to the great variation between individual animals. Addition of bST alone or cortisol alone decreased (non-significantly) lipogenesis in explants from both control and bST-treated animals.

In tissues from control animals, hormonal combination of insulin plus cortisol or insulin plus bST increased (P<0.05) lipogenesis and further addition of bST or cortisol decreased lipogenesis (P<0.05). In bST-treated animals, the above mentioned hormonal combinations changed lipogenesis in a similar manner, however, these changes were not significant.

The mean values of lipogenesis in freshly-prepared explants and cultured in the presence of different hormonal combinations and then incubated in the presence of NE or ISO are shown in Table 1. In freshly prepared explants NE decreased (P<0.05) the rate of fatty acid synthesis from 1.70 to 0.52 $nmol.mg^{-1} \cdot h^{-1}$ in bST-treated animals but not in controls. The effect of ISO was non-significant in both control and bST-treated animals. However, significant inhibition of lipogenesis by catecholamines was found in cultured explants from both control or bST-treated animals. The rate of acetate incorporation into fatty acids of cultured explants in the presence of NE in control (2.54±0.30 $nmol.mg^{-1}.h^{-1}$) and bST-treated (2.40±0.30 $nmol.mg^{-1}.h^{-1}$) animals was the same. However, the rate of lipogenesis in the presence of ISO was significantly (P=0.0097) lower in bST-treated (2.07±0.28 $nmol.mg^{-1}.h^{-1}$) than in control animals (3.21±0.28 $nmol.mg^{-1}.h^{-1}$). These results suggest that NE was equally effective in control and bST-treated animals whereas ISO was more effective in bST-treated animals than in the controls.

Discussion

The chronic administration of bST in growing castrated male goats increases the growth rate by 42 % and leads to a loss of lipids from omental adipose tissue by 44 % and decreases the proportion of fat in muscles by 30 % . This lipid loss in bST-treated animals resulted from the reduced rate of glucose-stimulated fatty acid synthesis (Škarda 1998).

However, it has been demonstrated that even in the absence of glucose, insulin stimulates protein synthesis from amino acids and inhibits the release of free fatty acids and glycerol from rat adipose tissue (Davidson 1987). The present study on goats has shown that chronic administration of bST *in vivo* and/or chronic (24 h) exposure of adipose explants to insulin, cortisol and bST *in vitro* have a profound influence on lipogenesis during acute incubation in a glucose-free buffer. This suggests that production of NADPH from the metabolism of acetate in a reaction catalyzed by NADP- isocitrate dehydrogenase which, in ruminants, furnishes one quarter of the NADPH necessary for lipogenesis (Bauman 1976) is sufficient to demonstrate the lipogenic effects of various hormones. A comparison of the lipogenesis of freshly-prepared explants incubated in a glucose-free buffer (present experiments) with those incubated in the presence of glucose (Škarda, 1998) showed that lipogenesis was 33-fold and 24-fold lower in tissues from control and bST-treated animals, respectively, when incubated in a glucose-free buffer.

Overall mean values of lipogenesis in a glucose-free buffer were consistently lower in bST-treated animals than in controls, whereas a pattern of changes in lipogenesis in cultures with different hormonal combinations was similar to that in explants incubated in the presence of glucose (Škarda 1998).

Bovine ST antagonized the ability of insulin to increase the rate of lipogenesis in the presence of cortisol in explants from controls and non-significantly in explants from bST-treated animals. A similar inhibitory effect of ST was observed in previous studies on adipose tissue from cows, goats and non-ruminants incubated in the presence of glucose (Etherton *et al.* 1987, Goodman 1993, Škarda 1998, 1999). In non-ruminants, there is a close relationship between lipogenesis and glucose metabolism so that lipogenesis decreased by ST can easily be explained by the inhibition of carbohydrate metabolic pathways proximal to acetyl CoA. In ruminants, however, this interpretation is not valid because acetyl CoA is synthesized from acetate (a product of rumen fermentation) *via* the acetyl CoA synthase reaction. This enzyme is more active in ruminant than in rat adipose tissue (Hanson and Ballard 1967) and together with the involvement of NADP-isocitrate dehydrogenase in NADPH production in ruminant adipose tissue could account for the ability of ruminant adipose tissue to synthesize fatty acids from acetate in the absence of glucose. In ruminants, ST can affect activities of enzymes (which are insulin- and cortisol-dependent) of the pentose cycle, isocitrate cycle and key lipogenic enzymes such as acetyl CoA synthase, acetyl CoA carboxylase and fatty acid synthase (Etherton and Bauman 1998).

The addition of cortisol alone to the culture medium decreased lipogenesis significantly in explants from controls and non-significantly in those from bST-treated animals (the rate of lipogenesis is already low in bST-treated animals). An inhibitory effect of cortisol was eliminated by the simultaneous addition of insulin similarly as it was demonstrated in the presence of glucose during acute incubation in goat (Škarda 1998, 1999) and bovine adipose tissue (Etherton *et al.* 1987). ST and glucocorticoids are hormones which act on lipogenesis as glucose and insulin antagonists. It has been shown that they reduce cellular glucose uptake and utilization. They act by inhibiting the glucose transport *per se*, perhaps by decreased synthesis of glucose transporters and by affecting glucose transporter subcellular trafficking (Goodman 1993, Weinstein *et al.* 1995, Zhao *et al.* 1996). Thus ST regulates glucose metabolism, insulin action and lipogenic enzyme activities in adipose tissue, thereby sparing glucose for increased growth or milk production. The mechanism that underlines the inhibition of lipogenesis by ST is not yet understood. Actinomycin D prevents the effect of ST on lipogenesis in sheep adipose tissue, suggesting that transcription of a mediator protein is required for the bST effect to occur (Borland *et al.* 1994). It was also shown that the antilipogenic, insulin-antagonistic effect of ST involves an increase in ornitine decarboxylase activity and an increase of both protein serine kinases and phosphatases, possibly including isoforms of protein kinase C and phosphatidyl choline-specific phospholipase C in mouse, rat and sheep adipose tissues (Gorin *et al.* 1990, Chou *et al.* 1990, Borland *et al.* 1994, Vernon 1996). On the other hand, insulin stimulation of phosphatidyl inositol-specific phospholipase is blocked by ST in plasma membranes from mouse adipose tissue (Roupas *et al.* 1991). Signal transduction pathways involved in transmitting the ST signal and mediating the metabolic action of ST also involve a decrease in the function of inhibitory (G_i) subunits of GTP-binding proteins (Roupas *et al.* 1991, Houseknecht and Bauman 1997).

In bST-treated goats, NE and ISO were equally effective whereas NE was more effective than ISO in controls. This contrasts with the results obtained in explants incubated in the presence of glucose (Škarda 1998, 1999). Nevertheless, the difference between pooled means of lipogenesis in the presence of NE in control and bST-treated animals was not significant. However, in the presence of ISO lipogenesis was lower in bST-treated than in control animals. These results are consistent with an increase in the number of β-adrenergic receptors in sheep adipocytes (Watt *et al.* 1991) and increased isoprenaline-stimulated lipolysis in bovine adipose tissue cultured in the presence of bST (Lanna *et al.* 1995).

In conclusion, a 24-h preculture of goat adipose explants in the presence of different hormones profoundly influences fatty acid synthesis in the subsequent acute incubation in a glucose-free buffer. Cortisol alone decreased lipogenesis from acetate, however, simultaneous addition of insulin eliminated this inhibition. Bovine ST antagonized the ability of insulin to increase the rate of lipogenesis in the presence of cortisol. Overall mean values of lipogenesis were lower in bST-treated animals than in the controls and isoprenaline decreased lipogenesis more in bST- treated animals than in controls. Thus ST treatment increases adipocyte responsiveness to β-adrenergic agonists.

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