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Hormonal Control of Net Glucose-Stimulated Lipogenesis During Transition From Brown to White Adipose Tissue in the Goat

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Summary

Perinatal (1-2 days of age) and one-month-old (24-32 days of age) male goats were used to investigate the effect of age and long-term culture (24 h) of perirenal and omental adipose explants in the presence of insulin, cortisol and bovine somatotropin (alone or in different combinations) on net glucose-stimulated lipogenesis (NGSL, i.e. the rate of lipogenesis in the presence of glucose minus the rate of lipogenesis in the absence of glucose) in the absence and in the presence of catecholamines in acute incubations (2 h). Mean values of NGSL in both freshly prepared and cultured explants were consistently lower in perinatal than in one-month-old goats. Cortisol alone decreased and combinations of insulin plus cortisol increased NGSL in perirenal explants of one-month-old animals. When perirenal explants from these one-month-old goats were cultured in the presence of insulin plus cortisol plus bovine somatotropin, the rates of lipogenesis were lower than those in cultures with insulin plus cortisol. No such effects of these hormones were noted in omental explants of both perinatal and one-month-old animals. In freshly prepared perirenal and omental explants, the rates of NGSL were inhibited by isoprenaline in tissues of both groups of animals and by noradrenaline in omental tissues of animals of the older group only. The mean values of NGSL in cultured explants of perinatal animals were not affected by noradrenaline. Isoprenaline inhibited NGSL in omental but not in perirenal tissue. In older animals the rates of NGSL were decreased by both noradrenaline and isoprenaline in perirenal and omental adipose tissues. Isoprenaline was more effective than noradrenaline in perirenal adipose tissue.

Key words

Goat • Ontogenesis • Adipose tissue • Lipogenesis • Hormones

Introduction

The development of adipose tissue has been divided into three phases: proliferation, differentiation and maturity. It appears that for ovine perirenal adipose tissue, all or at least most of the proliferative phase is complete by birth. This is followed in the immediate neonatal period by a phase of differentiation in which the brown adipocytes are transformed into white adipocytes. When this is complete, the adipocytes enter a mature phase, when further changes in the fat pad mass are due to changes in cell size (Vernon 1977).

Neonatal ruminants do not have a well-defined brown fat pad in the dorsal scapular region. However,

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microscopic and biochemical examinations of adipose tissue from various body regions show a typical brown adipose tissue structure and function on the first day of life (Thompson and Jenkinson 1970, Trayhurn *et al.* 1993). Although multivacuolar cells, brown adipose tissue-specific mitochondrial uncoupling protein and iodothyronine 5-deiodinase activity are present at birth in the adipose tissue of newborn ruminants, they disappear during the first days of postnatal life, indicating that there is a rapid transition from brown to white adipose tissue.

At birth, the body temperature must be maintained by mobilization and use of energy reserves. Sympathetic stimulation, in response to cold, increases lipolysis and blood flow through the fat depots to provide direct heat (non-shivering thermogenesis) and release free fatty acids as an energy substrate for shivering. Cold resistance of newborn animals is also increased by hormonally controlled fat deposition. Thermogenic effects of catecholamines are modulated by thyroid hormones, glucagon, somatotropin, ACTH, insulin and glucocorticoids (Janský 1995). Coordination of the secretion and action of these hormones is therefore required for perinatal survival and thus for the decrease of relatively high perinatal mortality in domestic farm animals.

In the present study, we have used acute incubation of freshly prepared and cultured adipose explants from perinatal and one-month-old goats to investigate the abilities of catecholamines to inhibit net-glucose stimulated lipogenesis (NGSL). In addition, the abilities of insulin, cortisol and somatotropin to stimulate or inhibit lipogenesis in chronically cultured adipose explants during transition from brown to white adipose tissue in goats were determined.

Methods

Animals and general procedures

The experiments were carried out on perinatal (aged 1-2 days) and one-month-old (aged 24-32 days) male goats of the Czech white breed. All animals were allowed to suckle and remained with their dams indoors under ambient temperature (10-17 °C) until they were killed. The great omentum and perirenal adipose tissues were aseptically removed and placed immediately into sterile phosphate buffered saline at about 38 °C. Explants were cultured (24 h) in a modified Waymouth's tissue culture medium (Škarda 1998) for 24 h in the presence of insulin (17 nmol.l^{-1}), cortisol (138 nmol.l^{-1}) and recombinant bovine somatotropin (bST; 4.5 nmol.l^{-1}) alone or in different combinations. Fresh adipose explants or cultured adipose explants (washed in saline at 38 °C) were transferred into 30 ml polyethylene flasks containing 3 ml of modified Krebs-Henseleit bicarbonate buffer and incubated for 2 h at 38 °C in an atmosphere of O_2/CO_2 (95:5; v/v) with reciprocal shaking at 90 strokes per min. Krebs-Henseleit bicarbonate buffer was either supplemented with 4 mmol.l^{-1} of sodium acetate (glucose-free buffer) (Škarda 1999a,c) or with sodium acetate and 3.5 mmol.l^{-1} of glucose (Škarda 1998, 1999b). To measure the responsiveness to catecholamines (inhibition of lipogenesis), explants were incubated in the presence or absence of either noradrenaline (NE; 10 μmol.l^{-1}) or isoprenaline (ISO; 10 μmol.l^{-1}). To measure the net glucose-stimulated lipogenic activity, the amount of (^{14}C)acetate incorporated into fatty acids of the explants in a modified Krebs-Henseleit buffer supplemented with sodium acetate alone was subtracted from the amount of acetate incorporated in the buffer supplemented with both sodium acetate plus glucose (Škarda 1999b). All incubations were run under conditions of linear incorporation of acetate and were terminated by cooling in an ice water bath. The explants were then collected, rinsed in cold saline and frozen at –20 °C until analyzed. All tissue incubations were carried out in triplicate.

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. When the labeling period was over, the explants were removed from plastic flasks, lipids were extracted and radioactivity was measured (Škarda *et al.* 1978). The rate of lipogenesis was expressed in terms of tissue protein (Škarda 1998) as nanomoles of acetate incorporated per mg protein per hour. The protein content of adipose tissue explants was determined after lipid extraction according to Lowry *et al.* (1951) with BSA as the standard.

Statistical methods

All results are expressed as means ± S.E.M. The rates of lipogeneses were evaluated by repeated ANOVA analysis (factors comprising hormones in culture, catecholamines on incubation and their interaction). All calculations were carried out by the GLM Procedure (SAS 1989). The effects of noradrenaline and isoprenaline in uncultured explants and in explants cultured in the presence of different hormones were compared by the paired t-test.

Table 1. Effect of chronic culture (24 h) in the presence of various hormones on the rate of net glucose-stimulated lipogenesis (NGSL) during acute incubation (2 h) in the presence or absence of catecholamines in adipose explants from perinatal goats aged 1-2 days.

Culture variables	Acetate incorporation into fatty acids ($nmol.mg^{-1}.h^{-1}$) Incubation variables		
	No catecholamines	NE	ISO
Perirenal adipose tissue			
Before culture:			
No hormones	3.90^{Aa}	3.40^{Aa}	1.61^{Ab}
After culture:			
No hormones	0.27^{Ba}	0.28^{Ca}	0.68^{ABa}
Insulin (I)	0.12^{Ba}	0.62^{BCab}	0.88^{ABb}
bST	1.04^{Ba}	0.56^{Ca}	0.35^{Ba}
Cortisol (H)	0.70^{Ba}	0.40^{Ca}	0.05^{Ba}
I + H	0.37^{Ba}	1.78^{Bab}	0.10^{Bac}
I + bST0.60^{Ba}	0.23^{Ca}	0.20^{Ba}	
I + H + bST	1.58^{Ba}	0.63^{BCb}	0.75^{ABab}
Overall mean after culture	0.67^{a}	0.64^{a}	0.43^{a}
Pooled S.E.M.	0.62	0.42	0.43
Omental adipose tissue			
Before culture:			
No hormones	23.91^{Aa}	26.29^{Aa}	6.33^{Ab}
After culture:			
No hormones	9.88^{BCa}	12.96^{Bab}	7.50^{Aac}
Insulin	13.18^{Ba}	12.11^{Ba}	10.89^{Aa}
bST	8.91^{BCa}	8.75^{Ba}	5.73^{Aa}
Cortisol	2.89^{Ca}	8.08^{Bb}	2.76^{Aa}
I + H	10.66^{BCa}	12.76^{Bab}	7.91^{Aac}
I + bST	8.99^{BCa}	8.38^{Ba}	4.76^{Aa}
I + H + bST	6.73^{BCa}	3.05^{Ba}	6.63^{Aa}
Overall mean after culture	8.75^{a}	9.44^{a}	6.60^{b}
Pooled S.E.M.	3.27	4.52	2.89

Perirenal adipose explants of six and omental adipose explants of five perinatal animals (killed 1-2 days after birth) were exposed before and after culture to (1-^{14}C)acetate (20 kBq.ml^{-1}) for 2-h period of incubation in the presence or absence of noradrenaline (NE) or isoprenaline (ISO) in Krebs-Henseleit buffer supplemented either with sodium acetate or sodium acetate plus glucose. The rate of lipogenesis in the presence of glucose minus rate of lipogenesis in the absence of glucose was taken as NGSL. Values are means ± pooled S.E.M. (standard error LS mean for paired values analyzed by ANOVA). Values within a column which do not have the same upper case superscript (A, B, C, D) differ significantly ($P<0.05$). Values within a row which do not have the same lower case superscript (a, b, c) differ significantly ($P<0.05$). Other details are described in the Methods.

Table 2. Effect of chronic culture in the presence of various hormones on the rate of NGSL during acute incubations in the presence or absence of catecholamines in perirenal and omental adipose explants from male goats aged 24-32 days.

Culture variables	Acetate incorporation into fatty acids (nmol.mg^{-1}.h^{-1}) Incubation variables		
	No catecholamines	NE	ISO
Perirenal adipose tissue			
Before culture:			
No hormones	80.28^{Aa}	78.51^{Aa}	27.19^{Ab}
After culture:			
No hormones	31.16^{BCa}	8.91^{Bb}	5.44^{BCb}
Insulin (I)	38.53^{BCa}	12.63^{B}	11.25^{Bb}
bST	30.80^{BCa}	9.34^{Bb}	3.77^{BCa}
Cortisol (H)	4.87^{Da}	4.93^{Ba}	1.03^{Cb}
I + H	51.81^{Ba}	18.78^{Bb}	7.01^{BCb}
I + bST	25.76^{CDa}	7.40^{Bb}	8.00^{BCb}
I + H + bST	14.03^{CDa}	7.28^{Ba}	6.32^{BCb}
Overall mean	28.14^{a}	9.90^{b}	6.12^{c}
Pooled S.E.M.	8.67	10.51	3.51
Omental adipose tissue			
Before culture:			
No hormone	47.39^{Aa}	13.98^{Ab}	4.62^{Ac}
After culture:			
No hormone	38.80^{Aa}	12.52	11.22^{Ab}
Insulin	34.93^{Aa}	10.91	12.65^{Ab}
bST	36.87^{Aa}	16.67^{Ab}	8.50^{Ac}
Cortisol	21.81^{Aa}	14.46^{Aa}	5.25^{Ab}
I + H	46.80^{Aa}	8.84^{Aa}	7.67^{Aa}
I + bST	34.90^{Aa}	15.07	13.06^{Aab}
I + H + bST	32.71^{Aa}	10.55^{Aa}	7.22^{Aa}
Overall m	35.26^{a}	12.72^{b}	9.37^{b}
Pooled S.E.M.	14.77	7.34	4.54

Perirenal adipose explants of six and omental adipose tissue explants of three male goats were incubated and cultured as it was described in the legend of Table 1.

Results

The rate of NGSL in freshly prepared adipose explants in 2-h incubations in a modified Krebs-Henseleit buffer was established to provide 1) the information on the effect of age (conversion of brown fat cells into white cells) on fatty acid synthesis *in vitro,* and 2) a measure of the maintenance of lipogenesis in subsequent incubations of explants cultured for 24 h in a modified Waymouth's medium in the presence of different hormones. Comparison of NGSL in freshly prepared perirenal and omental explants from perinatal animals with those cultured in the absence of hormones for 24 h showed a decrease ($P<0.05$) in the rate of NGSL after 24 h of culture (Table 1). In tissues from one-month-old animals NGSL was significantly lower ($P<0.05$) after 24 h of

culture in the perirenal explants but not in omental explants (Table 2). Mean values of NGSL in both freshly prepared and cultured explants were consistently lower in perinatal animals than in one-month-old goats (Tables 1 and 2).

NGSL 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. The addition of insulin alone, bST alone or insulin plus bST had no effect on the rate of NGSL in the perirenal and omental adipose tissue in animals of both age groups. Cortisol alone decreased ($P<0.05$) and combination of insulin plus cortisol increased ($P<0.05$) lipogenesis in perirenal explants from one-month-old animals. When perirenal explants from one-month-old goats were cultured in the presence of insulin plus cortisol plus bST, the rates of lipogenesis were lower ($P<0.05$) than those in cultures with insulin plus cortisol. No such effects of these hormonal combination on NGSL in omental explants of both age groups were observed.

In freshly prepared perirenal and omental adipose explants, the rates of NGSL were inhibited ($P<0.05$) by isoprenaline in tissues of both perinatal and one-month-old animals and by noradrenaline ($P<0.05$) in the omental tissue from older animals only. Thus isoprenaline was more ($P<0.05$) effective than noradrenaline (Tables 1 and 2).

The mean values of NGSL in explants cultured in the presence of different hormonal combinations and then incubated in the presence of noradrenaline or isoprenaline are shown in Tables 1 and 2. In perinatal animals the mean values of NGSL were not affected by noradrenaline. Isoprenaline inhibited ($P<0.05$) lipogenesis in the omental but not in the perirenal adipose tissue. In older animals, the rate of NGSL in the presence of catecholamines was decreased ($P<0.05$) in both perirenal and omental adipose tissues. Isoprenaline was more ($P<0.05$) effective than noradrenaline in the perirenal adipose tissue.

Discussion

At birth, the newborn organism is required to undergo major physiological adaptation to adjust to its new environment. At delivery, glucose released from glycogen reserves provides the immediate energy resource prior to the use of fat reserves or maternal milk. The ability to mobilize fatty acids has been considered to be critical, because animals in which it did not occur died (Randall 1992). Deposition of fat during the first few days of neonatal life increases the subsequent resistance to cold. Deposition and mobilization of glycogen and fat is under endocrine control, however, the relative importance of different hormones in different species is not clear. The adipose tissue from perinatal and one-month-old goats remains metabolically active and retains its ability to respond to hormones when maintained in culture in a modified Waymouth's tissue culture medium. However, the degree of maintenance was lower than that in adipose explants from 8-month-old goats (Škarda 1999b).

Perirenal adipose tissue of fetal lambs was found to be refractory to insulin, glucocorticoids and somatotropin and this refractoriness disappeared by birth (Vernon 1982). In the present study, insulin, cortisol and bovine somatotropin had no effect on the rate of lipogenesis in adipose explants of perinatal goats. This refractoriness to hormones disappeared in the perirenal adipose tissue by one month of age, but was still present in omental adipose explants. The responsiveness of the omental adipose tissue to hormones was, however, found in 4-month-old (Bartoš and Škarda 1970) and 8 month-old goats (Škarda 1998). The delay in the development of goat adipose tissue responsiveness to hormones could be explained by species differences between goat and sheep tissues. The differences in hormonal responsiveness between omental and perirenal adipose tissues demonstrate, in agreement with Bjorntorp *et al.* (1990), that adipose tissue is not a homogeneously developing and functioning entity, but exhibits quantitative differences in both fat accumulating and mobilizing activities in different regions.

In one-month-old goats, the rates of fatty acid synthesis expressed in terms of tissue protein were consistently higher in both perirenal explants (before culture a 20.6-fold, after culture a 42-fold increase) and omental explants (before culture 2-fold, after culture 4-fold increase) than those in explants from perinatal animals. The rates of fatty acid synthesis from acetate in omental explants from perinatal goats before culture exhibited a 6.1-fold increase and after culture a 13.1-fold increase compared to the perirenal explants. The higher rates of lipogenesis in omental than in perirenal adipocytes from 10-day-old lambs were also found by Vezinhet and Nougues (1977). Higher rates of lipogenesis in omental than in perirenal adipose tissue could reflect an earlier onset of the transformation of brown to white adipocytes in omental adipose tissue. Regional differences in the development of brown adipose tissue of lambs were demonstrated by Trayhurn

et al. (1993) using brown fat-specific uncoupling protein and its mRNA.

Infusion of noradrenaline into newborn lambs increased non-shivering heat production, but it disappeared as the tissue lost its characteristics of brown adipose tissue (Noble 1981). However, *in vitro* rates of NGSL were not inhibited by noradrenaline in both freshly prepared and cultured perirenal and omental explants from perinatal goats. It is possible that exposure of our newborn kids to low ambient temperature for the first 1-2 days before tissue explantation had already maximally stimulated brown fat by the sympathetic nervous system so that no further effect of noradrenaline could be expected. A lack of the noradrenaline effect on lipogenesis may also partly reflect the low lipogenic activity of adipocytes of newborn animals so that a further decrease is low. However, isoprenaline inhibited lipogenesis in explants of newborn animals and was more effective than noradrenaline in one-month-old animals. In our earlier experiments on tissues of 8-month-old castrated bST-treated male goats isoprenaline decreased the rate of lipogenesis in the presence of glucose more than noradrenaline, although in untreated animals noradrenaline and isoprenaline were equally effective (Škarda 1998, 1999b). It is probable that somatotropin secretion in perinatal and one-month-old animals is higher than that in older animals and one can thus expect a higher number of β-adrenergic receptors (Watt *et al.* 1991) to be present in young than in older animals. The higher activity of isoprenaline in young goats (present experiments) and in bST-treated older animals (Škarda 1998, 1999b) is due to the somatotropin-induced increased number of α_2-adrenergic receptors, which could limit the effects of noradrenaline but not those of isoprenaline (Vernon *et al.* 1995).

In conclusion, adipose explants of both perirenal and omental tissues from perinatal goats were refractory to insulin, cortisol and bovine somatotropin when maintained in tissue culture for 24 h. In perirenal explants this refractoriness to hormones disappeared by the age of one month, but was still present in omental explants. Uncultured explants of both perirenal and omental tissues from perinatal goats were refractory to noradrenaline, however, isoprenaline decreased NGSL significantly ($P<0.05$). In one-month-old goats isoprenaline was more effective than noradrenaline. Changes in the abilities of hormones to affect lipogenesis in the adipose tissue appear to be linked to developmental adaptations in adipose tissue function after birth. Further studies are required to clarify the effects of chronic maternal cold exposure and the effects of ambient temperature, at which the newborn kids are maintained, on hormone secretion and adipocyte hormone responsiveness during the transition from brown to white adipose tissue. The tissue ability to respond to hormones seems to be critical for survival of the neonate.

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Reprint requests

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