

Effects of photoperiod and feeding level on adipose tissue and muscle lipoprotein lipase activity and mRNA level in dry non-pregnant sheep

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The aim of the present study was to investigate the effects of photoperiod and feeding level on lipid metabolism in ovine perirenal and subcutaneous adipose tissues (AT) and in skeletal and cardiac muscles. Twenty dry non-pregnant ovariectomised ewes were divided into two groups and subjected to either 8 h or 16 h light/d, and underfed at 22 % energy requirements for 7 d. Half of the ewes in each group were slaughtered and the remaining ewes were refed at 190 % energy requirements for 14 d, until slaughtering. Refeeding increased (2.6–4.3-fold) malic enzyme (ME), fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH) and glycerol-3-phosphate dehydrogenase (G3PDH) activities in subcutaneous AT as well as lipoprotein lipase (LPL) activity in perirenal (3.5-fold) and subcutaneous (10-fold) AT and to a lesser extent (1.4-fold) in the skeletal *longissimus thoracis* and cardiac muscles. Moreover, variations of LPL mRNA level followed variations of LPL activity: refeeding increased perirenal AT- and cardiac muscle-mRNA levels (7.4- and 2-fold respectively). The main finding of this study is that, for a given level of food intake, long days (compared with short days) increased the LPL activity in the *longissimus thoracis* muscle and, in refed ewes, the activities of LPL and ME in subcutaneous AT. Furthermore, long days increased LPL mRNA level in cardiac muscle and perirenal AT. Thus, our results show that there are direct effects of photoperiod on sheep AT lipogenic potential, as well as on muscle LPL activity, which are not caused by changes in nutrient availability.

Photoperiod: Lipoprotein lipase: Adipose tissues: Muscles: Sheep

Many species of mammals undergo pronounced seasonal fluctuations, primarily for their reproduction but also for coat growth, food consumption, body metabolic rate and body weight (Lincoln & Richardson, 1998; Mercer, 1998). Such fluctuations have been shown to be mainly under the control of daylength (photoperiod). Interestingly, seasonal changes in adipose tissue (AT) metabolism have been observed in wild ruminants (Abbott *et al.* 1984; Larsen *et al.* 1985*a,b*). Among livestock, the sheep is a well-known seasonal breeder (Ortavant *et al.* 1988) and the possibility that its AT metabolism varies with season has been suggested (Vernon *et al.* 1986). However, the seasonal variations in daylength are accompanied by fluctuations in feed resources and voluntary food intake of the animals. This is why we investigated sheep in a trial designed to avoid any indirect effects of the photoperiod on AT and

muscle metabolism that could be due to changes in food intake.

It was shown in a previous study that photoperiod affected the activity of lipogenic enzymes (i.e. fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), and glycerol-3-phosphate dehydrogenase (G3PDH)) in perirenal AT (Bocquier *et al.* 1998). The aim of the present study was to look for such an effect on lipoprotein lipase (LPL), the key enzyme mediating the uptake of fatty acids from circulating lipid-rich lipoproteins by peripheral tissues (Borensztajn, 1987). This enzyme makes fatty acids available in AT mainly for storage in the fat cell lipid droplet, while in muscle it generates fatty acids that are predominantly used as an energetic substrate. It has been shown that AT-LPL activity responds to photoperiod with regional differences between

Abbreviations: AT, adipose tissue; FAS, fatty acid synthase; G3PDH, glycerol-3-phosphate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; LPL, lipoprotein lipase; ME, malic enzyme.

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AT sites in male Siberian hamsters (Bartness *et al.* 1989; Bartness, 1995). We therefore examined the respective effects of different light treatments (short *v.* long daylength exposure) and feeding level (underfed *v.* refed) on LPL activity in an internal AT site, the perirenal AT, and an external site, the subcutaneous AT of strictly pair-fed ewes. Moreover, the effects of these treatments were also studied on LPL activity in two muscles with different metabolic characteristics: the glycolytic *longissimus thoracis* skeletal muscle and the oxidative cardiac muscle. LPL activity measurements were completed by LPL mRNA assays in perirenal AT and cardiac muscle. In addition, the activity of other lipogenic enzymes were also measured in the subcutaneous AT, in order to compare them with responses observed previously in perirenal AT.

Materials and methods

Animals and experimental procedure

Twenty dry non-pregnant adult Lacaune ewes had previously been ovariectomized in order to avoid any putative indirect effect of photoperiod via changes in the secretion of sexual hormones, which are potential modulators of AT metabolism (Chilliard, 1987). The animals were placed, during a 3 week pre-experimental period, in individual pens on a hay–concentrate (70:30, w/w) diet providing 123 (SD 2) % energy requirements calculated on the basis of 0.4 MJ metabolizable energy/d per kg body weight 0.75 (Institut National de la Recherche Agronomique, 1989). Thereafter, ewes were randomly arranged according to their body weight and body condition score (a score related to the subcutaneous fat thickness), in a 2 × 2 factorial design (five ewes per treatment) in which both the level of feeding (underfeeding *v.* refeeding) and daylength (short *v.* long) were controlled. Light treatment started in January (natural light period of approximately 9 h/d) and ewes were subjected either to short (S, 8 h light/d, fluorescent bulb 500 lx) or long (L, 16 h light/d, same intensity) constant daylength. In short-day treatment, light was switched on at 08.00 hours and switched off at 16.00 hours while long-days started at 04.00 hours and finished at 20.00 hours. After 3 weeks during which ewes received 123 (SD 2) % energy requirements, all ewes were given a restricted amount of food (hay–straw (66:34, w/w) diet) corresponding to 22 (SD 1) % energy requirements for 7 d. Thereafter five short-day ewes and five long-day ewes were slaughtered at the end of the underfeeding period, and the remaining ewes were refed (hay–concentrate (45:55, w/w) diet, with pair-feeding according to actual feed intake across photoperiodic treatments) for 14 d until slaughter, when their level of intake met 190 (SD 2) % energy requirements. The concentrate was: (g/kg) corn 190, sugar beet pulp 300, soyabean meal 416, molasses 20, fish meal 50, vitamins and minerals 24. Vitamin–mineral premix (20 g/d) was added to the feed of each group (minerals, (g/kg): Ca 150, P 100, Mg 20, Na 30, S 10; trace elements (mg/kg): Zn 8000, Mn 6000, I 50, Co 10, Se 10; vitamins (mg/kg): retinyl acetate 86, cholecalciferol 1.25, α -tocopherol 134, thiamine hydrochloride 21). Diet for the underfed groups was offered at 10.00 hours, and that of the

Table 1. Ingredients, energy and protein ingested daily by the underfed and refed ewes

(Mean values with standard errors of the means for ten ewes per group)

Feeding level*...	22		190	
	Mean	SEM	Mean	SEM
DM (g/d)	289 ^a	11	1748 ^b	68
Organic matter, (g/d)	264 ^a	10	1589 ^b	61
Crude protein (g/d)	26 ^a	1	370 ^b	13
Crude fibre (g/d)	105 ^a	5	329 ^b	15
Energy (MJ ME/d)	2.1 ^a	0.1	18.9 ^b	1.5
Protein (g PDI/d)	19 ^a	1	225 ^b	8

ME, metabolizable energy; PDI, protein digestible in the intestine (Institut National de la Recherche Agronomique, 1989).

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P < 0.01$).

* Percentage of maintenance requirements.

refed group was divided into two equal portions given at 10.00 hours and 15.00 hours. Ewes had free access to drinking water. Offered feeds and refusals were recorded daily so as to calculate daily intakes of each animal (Table 1).

Ewes were slaughtered by exsanguination and samples of perirenal and subcutaneous AT and cardiac and *longissimus thoracis* muscles were rapidly excised and either placed immediately at +37°C for adipocyte volume determination or frozen in liquid N₂ pending measurements of DNA content, LPL and lipogenic activities and LPL mRNA levels.

All experimental procedures involving the use of animals were conducted after approval by the Animal Care and Use Committee of Institut National de la Recherche Agronomique (INRA).

Adipose tissue and muscle measurements

The FAS, G6PDH, ME and G3PDH activities were assayed spectrophotometrically in subcutaneous AT as described previously (Chilliard *et al.* 1991).

LPL activity was measured in subcutaneous and perirenal AT and in cardiac and *longissimus thoracis* muscles using an artificial emulsion containing [³H]-triolein after a detergent (Deoxycholate-Nonidet P40, Sigma Chemical, Saint-Quentin-Fallavier, France) extraction procedure (Faulconnier *et al.* 1994).

Enzyme activity was expressed either on a tissue weight basis, i.e. nmol released fatty acids (LPL) or nmol reduced (G6PDH, ME) or oxidised (FAS, G3PDH) nucleotides per min and per g tissue, or on a cellular basis, i.e. per 10⁶ adipocytes for AT or per mg DNA for muscles, after measurement of adipocyte volume (Chilliard *et al.* 1991) or muscle DNA content (Hocquette *et al.* 1998).

The levels of LPL mRNA in perirenal AT and cardiac muscle were quantified by real time quantitative reverse transcriptase – polymerase chain reaction, using the fluorescent TaqMan methodology on 7700 Sequence Detector System (PE Applied Biosystems, Courtaboeuf, France) as described by Bonnet *et al.* (2000). The levels of LPL mRNA were related to the levels of cyclophilin mRNA, a housekeeping gene, measured by real time reverse transcriptase – polymerase chain reaction.

Table 2. Effects of feeding level and photoperiod on lipogenic activities in ovine subcutaneous adipose tissue (Mean values with standard errors of the means for five ewes per group)

Feeding level*...	22				190				Statistical significance of effects ($P <$)		
	S		L		S		L				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	F	P	F×P
FAS†											
U/g tissue	67 ^a	13	113 ^a	34	354 ^b	56	372 ^b	38	0.01	0.42	0.72
U/10 ⁶ adipocytes	36 ^a	9	60 ^a	23	200 ^b	24	224 ^b	28	0.01	0.29	0.99
G6PDH†											
U/g tissue	291 ^a	43	279 ^a	48	1054 ^b	81	1125 ^b	222	0.01	0.81	0.74
U/10 ⁶ adipocytes	147 ^a	14	147 ^a	37	610 ^b	45	663 ^b	140	0.01	0.72	0.73
ME†											
U/g tissue	79 ^a	15	99 ^a	6	203 ^{bc}	25	269 ^c	45	0.01	0.13	0.40
U/10 ⁶ adipocytes	41 ^a	9	51 ^a	8	117 ^b	13	158 ^c	20	0.01	0.08	0.25
G3PDH†											
U/g tissue	5659 ^a	887	8557 ^{ab}	1909	15905 ^c	1935	17907 ^c	2559	0.01	0.21	0.82
U/10 ⁶ adipocytes	3032 ^a	536	4471 ^a	1336	9128 ^b	906	10377 ^b	871	0.01	0.17	0.92

S, 8 h light/d, L, 16 h light/d; F, feeding level; P, photoperiod; F×P, feeding level×photoperiod interaction; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme; G3PDH, glycerol-3-phosphate dehydrogenase.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* Percentage of maintenance requirements. For details of diets see Table 1.

† One unit (u) of activity is defined as 1.0 nmol reduced (G6PDH, ME) or oxidised (FAS, G3PH) nucleotides/min.

Statistical analysis

Data presented in Tables 1, 2 and 4 and in Fig. 1 were analysed with the general linear models procedure of the Statistical Analysis Systems program (version 6; 1987; SAS Institute Inc., Cary-NC, USA). The first analysis was done according to a 2×2 factorial design testing the effects of feeding level (ten refed v. ten underfed ewes) and photoperiod (ten short v. ten long days), together with the significance of refeeding×photoperiod interaction. The second analysis was performed comparing the four groups of five ewes. Differences between groups were tested using the Student-Newman-Keul's test. Data presented in Table 3 were made using the pairwise Student's *t* test for paired data. Values were considered to be significantly different if *P* values were less than 0.01 or 0.05.

Results

Lipogenic enzyme activities in subcutaneous adipose tissue

The FAS, ME, G6PDH and G3PDH activities, expressed per adipocyte, were significantly higher ($P < 0.01$) in refed than in underfed ewes (Table 2). The magnitude of the

increase was similar for FAS and G6PDH (+330 % activities, and less for ME and G3PDH (+200 and +160 % respectively) activities. Similar trends were observed when these activities were expressed per g AT.

Furthermore, the ME activity, expressed per adipocyte, tended ($P < 0.08$) to be greater in subcutaneous AT of ewes subjected to long photoperiod (Table 2). The response was more significant and of larger magnitude in refed (+35 %, $P < 0.05$) than in underfed ewes (+24 %). There was a positive but not significant effect of long daylength for FAS and G3PDH activities. The G6PDH activity was unaffected by the photoperiod (Table 2). Similar trends were observed when these activities were expressed per g AT.

Lipoprotein lipase activity in adipose tissues and muscles

LPL activity, expressed per adipocyte, was significantly ($P < 0.01$) greater in perirenal than in subcutaneous AT (Table 3). This effect was more marked in underfed (+400 %, $P < 0.01$) than in refed (+80 %, $P < 0.02$) ewes. This activity, expressed per mg DNA, was significantly ($P < 0.01$) greater in cardiac than in *longissimus thoracis*

Table 3. Lipoprotein lipase activities according to anatomical sites in the ewes (Mean values with standard errors of the means for ten ewes per group)

Feeding level*	Adipose tissues (U/10 ⁶ adipocytes)†				Muscles (U/mg DNA)†			
	Perirenal		Subcutaneous		Cardiac		<i>Longissimus thoracis</i>	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
22	170 ^A	10	34 ^B	5	131 ^A	7	26 ^B	2
190	596 ^a	102	340 ^b	41	173 ^A	14	38 ^B	5

^{A,B,a,b} Mean values within a row, and within adipose or muscle tissue, with unlike superscript letters were significantly different ($P < 0.01$ and $P < 0.05$ respectively).

* Percentage of maintenance requirements. For details of diets see Table 1.

† One unit (u) of activity is defined as 1.0 nmol released fatty acids/min.

Table 4. Effects of feeding level and photoperiod on lipoprotein lipase activities in ovine adipose and muscle tissues (Mean values with standard errors of the means for five ewes per group)

Feeding level*...	22				190				Statistical significance of effects ($P <$)		
	S		L		S		L				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	F	P	F × P
Adipose tissues											
Perirenal											
U/g tissue†	153 ^a	10	194 ^a	14	698 ^b	85	647 ^b	66	0.01	0.92	0.40
U/10 ⁶ adipocytes†	158 ^a	10	184 ^a	10	589 ^b	93	604 ^b	111	0.01	0.78	0.94
Subcutaneous											
U/g tissue†	64 ^a	2	67 ^a	2	460 ^b	67	696 ^c	71	0.01	0.03	0.03
U/10 ⁶ adipocytes†	34 ^a	4	35 ^a	6	264 ^b	35	415 ^c	47	0.01	0.02	0.02
Muscles											
Cardiac											
U/g tissue†	300 ^a	18	297 ^a	15	435 ^b	29	366 ^{ab}	36	0.01	0.19	0.22
U/mg DNA†	133 ^a	8	129 ^a	5	187 ^b	12	160 ^{ab}	16	0.01	0.18	0.33
<i>Longissimus thoracis</i>											
U/g tissue†	52 ^a	6	71 ^{ab}	3	80 ^{ab}	9	92 ^b	13	0.01	0.08	0.74
U/mg DNA†	23 ^a	2	30 ^{ab}	2	33 ^{ab}	3	43 ^b	8	0.02	0.06	0.80

S, 8 h light/d, L, 16 h light/d; F, feeding level; P, photoperiod; F × P, feeding level × photoperiod interaction.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* Percentage of maintenance requirements. For details of diets see Table 1.

† One unit (u) of activity is defined as 1.0 nmol released fatty acids/min.

muscle, to the same extent in either underfed (+390 %) or refed (+350 %) ewes. Similar trends were observed when LPL activity was expressed per g tissue.

Refeeding significantly ($P < 0.01$) increased LPL activity (+900 %) in subcutaneous AT and to a lesser extent (+250 %) in perirenal AT (Table 4). Cardiac and *longissimus thoracis* muscle LPL activities, expressed per mg DNA, were also significantly ($P < 0.01$ and $P < 0.02$ respectively) modulated by feeding level, in the same direction but with smaller amplitudes than in AT. Hence, refeeding increased by +40 % these activities in the two muscles. Similar trends were observed when LPL activity was expressed per g tissue.

LPL activity in subcutaneous AT, expressed per adipocyte, was significantly ($P < 0.02$) modulated by photoperiod, but differently according to the feeding level (feeding level × photoperiod interaction, $P < 0.02$) (Table 4). Hence, this activity was significantly higher (+57 %, $P < 0.05$) in AT of refed ewes exposed to long rather than to short days. However, this effect of photoperiod was not apparent in underfed ewes, whose AT-LPL activity was very low. LPL activity in *longissimus thoracis* muscle, expressed per mg DNA, tended also ($P < 0.06$) to be modulated by photoperiod. This activity was indeed 30 % greater in ewes exposed to long rather than to short days whatever the feeding level. However, the daylength did not affect perirenal AT and cardiac muscle LPL activities, in either underfed or refed ewes. Similar trends were observed when LPL activity was expressed on a per g basis.

Lipoprotein lipase mRNA levels in perirenal adipose tissue and cardiac muscle

The LPL mRNA levels were significantly ($P < 0.01$) modulated by feeding level in perirenal AT and cardiac muscle (Fig. 1). The LPL mRNA levels changed in the same direction in these two tissues during refeeding,

although these effects were more marked in perirenal AT (+640 %) than in cardiac muscle (+100 %).

Long days (*v.* short days) tended ($P < 0.1$) to increase the level of perirenal AT-LPL mRNA in underfed ewes (+45 %) (Fig. 1). This effect was significant and of larger magnitude (+162 %, $P < 0.05$) in refed ewes, owing to the trend ($P < 0.12$) towards a refeeding × photoperiod interaction. Long days also tended ($P < 0.06$) to increase the level of cardiac muscle LPL mRNA to the same extent in both underfed (+66 %) or refed (+60 %) ewes.

Discussion

Effect of feeding level on activities of lipogenic enzymes and lipoprotein lipase

The increase in subcutaneous AT lipogenic enzyme activities with refeeding is consistent with previous results of Bocquier *et al.* (1998) who observed, in the same experimental conditions, that refeeding also increased the FAS, G6PDH, G3PDH and ME activities in perirenal AT by factors of 4.8, 3.8, 2 and 2.2 respectively. The magnitude of the lipogenic enzyme variations seems to be related to the duration but also to the level of refeeding. Indeed, in lambs fasted for 8 d, refeeding did not change the FAS, G6PDH and ME activities in subcutaneous AT (Ingle *et al.* 1973) probably because they were refed at only 80 % energy requirement for 8 d, whereas our ewes were refed at approximately 190 % for 14 d.

The present study confirms that refeeding regulates ovine LPL activity in the same direction in skeletal muscle as in cardiac muscle and AT, although with a lesser amplitude in muscles, in agreement with recent observations in underfed–refed ewes (Bonnet *et al.* 2000) and cows (Faulconnier *et al.* 1999). Furthermore, feeding-level effects were due, at least in part, to a pretranslational regulation (Fig. 1) as in rodents (Ladu *et al.* 1991). The

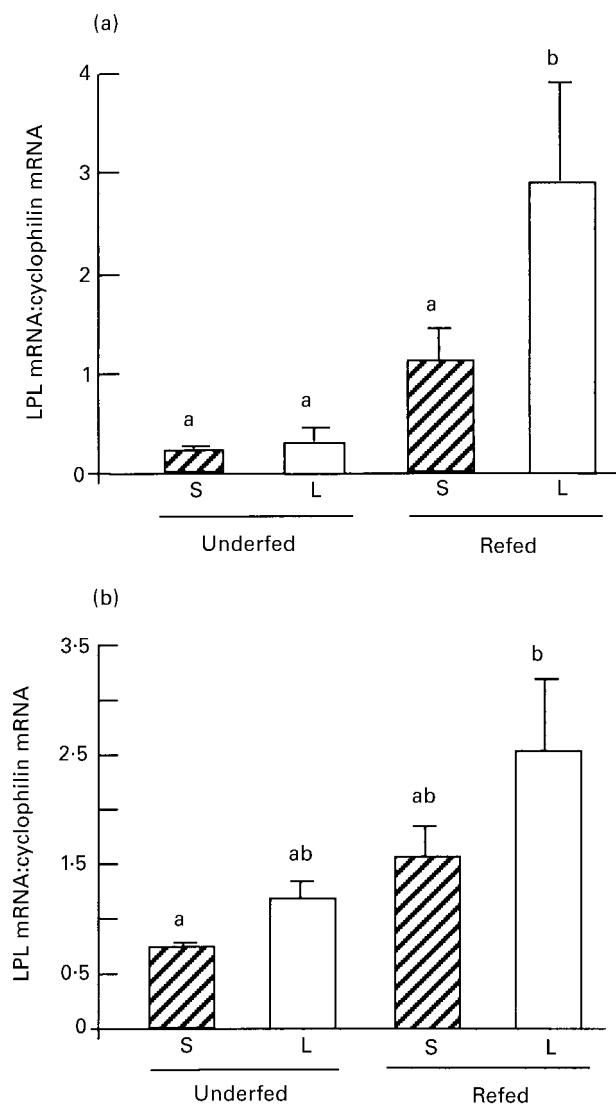


Fig. 1. Effect of feeding level and photoperiod (S, 8 h light/d; L, 16 h light/d) on the lipoprotein lipase (LPL) mRNA:cyclophilin mRNA ratio in (a) perirenal adipose tissue and (b) cardiac muscle. Values (arbitrary units) are means for five ewes with their standard errors represented by vertical bars. For details of diets see Table 1. ^{a,b}Mean values with unlike superscript levels were significantly different ($P < 0.05$). (a), statistical significance of effect of: refeeding $P < 0.01$, photoperiod $P < 0.1$, refeeding \times photoperiod $P < 0.12$; (b), statistical significance of effect of: refeeding $P < 0.01$, photoperiod $P < 0.06$, refeeding \times photoperiod $P < 0.52$.

upregulation of AT-LPL activity by refeeding is consistent with previous observations in several AT sites of adult (Chilliard *et al.* 1979; Bonnet *et al.* 2000) and growing (DiMarco *et al.* 1981) ruminants, and rats (Sugden *et al.* 1993; Bergö *et al.* 1996). The similar range of variation for plasma insulin level and AT-LPL activity and mRNA levels (Bonnet *et al.* 2000) suggests that dietary action could be mediated at least in part by insulin in sheep, as in monogastric species (Eckel, 1987). The observed upregulation by refeeding of muscle LPL activity, with the same relative increase in cardiac and skeletal muscles, contrasts with the decrease or the lack of variation observed in

skeletal or cardiac muscles, respectively, after the refeeding of starved rats (Quig *et al.* 1983; Sugden *et al.* 1993). These differences between species in the nutritional regulation of muscle LPL activity may be either of experimental origin (level or length of refeeding) or, more probably, related to the peculiarities of nutrient digestion and absorption, and liver lipogenesis, in ruminant *v.* rodent species (Bonnet *et al.* 2000). Although previous studies have analysed the possible effects of plasma glucose, glucagon, glucagon:insulin ratio and catecholamines (Borensztajn, 1987), the factors which mediate dietary action on muscle-LPL gene expression and activity has not been identified in any animal species.

LPL activity was much greater in perirenal than in subcutaneous AT, in keeping with results reported for cattle (Hocquette *et al.* 1998), goat (Chilliard *et al.* 1981) and rat (Cruz & Williamson, 1992) AT. Furthermore, LPL activity was greater in cardiac (oxidative) than in skeletal (glycolytic) muscle as described in bovine (Hocquette *et al.* 1998) and rat (Ladu *et al.* 1991; Ong *et al.* 1994) muscles.

Effect of photoperiod on lipogenic enzyme and lipoprotein lipase activities

Several studies in wild ruminants and rodents have shown that seasonal body weight changes are mainly due to seasonal cycles in fat deposition and AT lipogenic activity, the non-fat body mass remaining fairly constant (Reimers *et al.* 1982; Wade & Bartness, 1984). Reindeer, which inhabit arctic areas, are able to deposit large amounts of body fat during late summer and autumn, whereas fat reserves are mobilised during the winter period, due to alterations in lipogenic enzyme activity and in the lipolytic activation system (Larsen *et al.* 1985a,b). Another case is the white-tailed doe fawn, in which a seasonal (autumn in MI, USA) or artificial decrease in daylength increased body fat weight, which was explained in part by changes in AT lipogenic activity, but was not clearly related to *in vitro* adrenergic lipolysis (Abbott *et al.* 1984). Daylength also has important effects on white AT mass in both Syrian and Siberian hamsters, even though in one species short days increased (Syrian) and in the other decreased (Siberian) the weight of body fat (Wade & Bartness, 1984; Bartness, 1995). In keeping with these observations, short days decreased total LPL activity in white AT of Siberian hamsters (Bartness *et al.* 1989; Bartness, 1995).

In domestic sheep, it was observed that the rate of AT fatty acid synthesis and the activity of the LPL were increased 5.8- and 2.3-fold respectively, between October and May in Scotland, UK (Vernon *et al.* 1986). The activity of AT 5'-nucleotidase also increased during the spring in unmated sheep (Vernon & Taylor, 1986).

However, in these previous animal models, changes in AT metabolism responded similarly to changes in voluntary food intake and/or accompanying changes in plasma insulin and energy metabolites that were available for lipogenesis. For this reason it is not possible to know whether there is a direct effect of photoperiod (or other seasonal factors) on AT metabolism, besides indirect

effects due to changes in food intake or in the secretion of several metabolic or sexual hormones.

The present study shows that the ME activity of subcutaneous AT was (refed ewes) or tended to be (underfed ewes) increased by long daylength. This result is in agreement with data from Bocquier *et al.* (1998) who reported, in the same experimental conditions, that ME activity was significantly increased in the perirenal AT of ewes subjected to a long photoperiod, with a higher response in refed ewes (+41 and +18 % in refed and underfed ewes respectively). Similar trends were also observed in subcutaneous AT for FAS and G3PDH activities (Table 2), although the increase in these two lipogenic enzymes with long days did not reach statistical significance, probably due to the small number (n 5) of ewes per group. These increases in lipogenic activities with long days are consistent with the fact that plasma non-esterified fatty acids decreased in the ewes adapted to long daylength (Bocquier *et al.* 1998).

Moreover, the present study shows that long days significantly increase LPL activity in subcutaneous AT as well as in the *longissimus thoracis* skeletal muscle, and tend to increase perirenal AT and cardiac muscle-LPL mRNA levels, but without changing LPL activity in the latter two tissues. It thus appears that, in the ewes, long days increased LPL activity more in externally than in internally located tissues while the contrary was observed in male Siberian hamster AT (Bartness *et al.* 1989). Indeed, in the hamster, the long days decreased LPL activity more in internally than in externally located AT. This effect could be related to a difference in catecholaminergic innervation between anatomical AT (Youngstrom & Bartness, 1995) since white AT-LPL activity is regulated by catecholamines (Chiappe de Cingalani *et al.* 1996). In another respect, the fact that skeletal muscle was more sensitive to photoperiod changes than cardiac muscle may be related to a higher priority for maintaining in all circumstances the availability of nutrients for cardiac function.

In our present study, the effects of photoperiod were not driven by nutrient supply, since food intake was identical between groups of ewes, and because plasma insulin and metabolites (glucose, acetate, β -hydroxybutyrate, triacylglycerol), which all responded significantly to the feeding level and thus may have indirect effects on AT lipogenesis, remained stable between light treatments (Bocquier *et al.* 1998). Hence, our work provides evidence that photoperiod directly affects ovine AT lipogenic potential as well as muscle LPL activity.

The mechanisms through which photoperiod influences lipid metabolism remain to be unravelled (Chilliard & Bocquier, 2000). Peripheral effects of pineal melatonin, whose secretion is increased by short days (Chemineau *et al.* 1996), are plausible since specific binding sites have been described in Siberian hamster brown AT (Le Gouic *et al.* 1997) and in the heart of birds (Pang *et al.* 1993). However, the putative functionality of these receptors remains to be shown. Moreover, the *in vitro* incubation of white fat cells from several species with melatonin at doses lower than 300 pM has no effect on lipolysis or lipogenesis (Ng & Wong, 1986). However, physiological melatonin

(100 pM) inhibited *in situ* lipogenesis in the rat white inguinal AT by blocking fatty acid transport via a melatonin receptor-mediated mechanism (Blask *et al.* 1999). Another recent report has shown that melatonin treatment decreased rat body weight, intra-abdominal adiposity, plasma insulin and plasma leptin, while increasing subcutaneous fat, core body temperature and plasma corticosterone level (Wolden-Hanson *et al.* 2000). Several of the changes induced by melatonin (increase of core body temperature or plasma corticosterone, decrease of plasma insulin) could contribute to decrease LPL activity (Eckel, 1987) during short days.

The variations of plasma prolactin could also be involved in the photoperiod effect: lipomobilisation increases in short days when prolactinaemia is low, and lipogenic activities increase in long days when prolactinaemia is high (Bocquier *et al.* 1998). However, this hormone is probably not directly involved in AT response to daylength in sheep because their AT appears to lack prolactin receptors (Emane *et al.* 1986; Vernon, 1989). There could be, however, indirect effects of prolactin *in vivo* via the secretion of a putative hepatic factor, such as synlactin (English *et al.* 1990).

The complexity of the transfer of photoperiodic information is shown by the fact that hypothalamo-pituitary disconnection in rams suppresses the cyclical effects of photoperiod changes on α -melanocyte-stimulating hormone, β -endorphin, adrenocorticotrophic hormone, luteinizing hormone, follicle-stimulating hormone, feed intake and body weight, but does not suppress the cyclical changes in prolactin (Lincoln & Richardson, 1998). This means that photoperiod-driven signals act on pituitary hormones either via the hypothalamus by neuro-hormonal pathways (α -melanocyte-stimulating hormone, β -endorphin, adrenocorticotrophic hormone, luteinizing hormone, follicle-stimulating hormone) or directly on the pituitary secretion (prolactin), and that prolactin is not involved in body weight changes. Moreover, it has been shown that adrenocorticotrophic hormone acts on mouse adipocytes by decreasing both leptin release and leptin mRNA level, and increasing lipolysis (Renz *et al.* 2000), and on rat AT by decreasing LPL activity (Baggen *et al.* 1987). The increase in adrenocorticotrophic hormone levels by short days (Lincoln & Richardson, 1998) could therefore be one factor related to the decrease in LPL activity in the ewes by short days. It could also be related to the fact that short days decreased plasma leptin concentration and AT-leptin mRNA level in the ewes, while lipolysis increased (Bocquier *et al.* 1998). On the other hand, it has been proposed that, in rodents, the seasonal cycle in body stores is controlled by changes in the circadian rhythms of corticosteroids, prolactin and insulin (Meier & Cincotta, 1996). It thus appears necessary to analyse carefully the circadian variations of these three hormone concentrations in sheep, and the sensitivity of AT to their effects, and whether they are changed by variations in daylength.

Whatever the mechanism involved, the effects of photoperiod on AT and muscle metabolic activities seem to coincide with annual fluctuations of food resources (Chilliard & Bocquier, 2000). Indeed, the ability of AT to deposit fat and of muscles to use plasma triacylglycerol are

increased by long days, i.e. in phase with the natural seasonal increase in food availability. Conversely, mobilisation of body fat is increased by short days, i.e. during winter when less food is available. Moreover, the increase in muscle LPL activity in long days could reflect an adaptation for increasing muscle protein deposition and/or physical activity and foraging, since this enzyme controls in part the entry of energy fuel, such as fatty acids, into muscle cells. These results suggest that adult sheep have kept the ability to anticipate seasonal changes in feed resources, since their AT and muscle metabolism is sensitive to changes in daylength even when food intake is kept constant. It remains to be known how simultaneous changes in the lighting regimen and food availability can be manipulated in the long term in order to control changes in body composition in these animals.

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References

- Abbott MJ, Ullrey DE, Ku PK, Schmitt SM, Romsos DR & Tucker HA (1984) Effect of photoperiod on growth and fat accretion in white-tailed doe fawns. *Journal of Wild Management* **48**, 776–787.
- Baggen MG, Jansen H, Lammers R, Verschoor L & Birkenhager JC (1987) Disparate effects of ACTH (1–24) and corticosterone on lipoprotein lipase in rat adipose tissue. *Journal of Endocrinology* **114**, 369–372.
- Bartness TJ (1995) Short-day-induced depletion of lipid stores in fat pad- and gender-specific in Siberian hamsters. *Physiology and Behaviour* **58**, 539–550.
- Bartness TJ, Hamilton JM, Wade GN & Goldman BD (1989) Regional differences in fat pad responses to short days in Siberian hamsters. *Integrative Comparative of Physiology* **26**, R1533–R1540.
- Bergö M, Olivecrona G & Olivecrona T (1996) Diurnal rhythms and effects of fasting and refeeding on rat adipose tissue lipoprotein lipase. *American Journal of Physiology* **271**, E1092–E1097.
- Blask DE, Dauchy RT & Sauer LA (1999) Physiological melatonin inhibits lipogenesis and fasting-induced lipolysis in the inguinal fat pad perfused *in situ* in the rat. In *Proceedings of the 81st Annual Meeting of the Endocrine Society*, P2–208, p. 324. San Diego, CA.
- Bocquier F, Bonnet M, Faulconnier Y, Guerre-Millo M, Martin P & Chilliard Y (1998) Effects of photoperiod and feeding level on perirenal adipose tissue metabolic activity and leptin synthesis in the sheep. *Reproduction Nutrition Development* **38**, 489–498.
- Bonnet M, Leroux C, Faulconnier Y, Hocquette JF, Bocquier F, Martin P & Chilliard Y (2000) Lipoprotein lipase activity and messenger RNAs are up-regulated by refeeding in adipose tissue and cardiac muscle of sheep. *Journal of Nutrition* **30**, 749–756.
- Borensztajn J (1987) Heart and skeletal muscle lipoprotein lipase. In *Lipoprotein Lipase*, Chapter 5, pp. 133–148 [J Borensztajn, editor]. Chicago, IL: Evener Publishing.
- Chemineau P, Malpoux B, Pelletier J, Leboeuf P, Delgado JA, Deletang F, Pobel T & Brice G (1996) Emploi des implants de mélatonine et des traitements photopériodiques pour maîtriser la reproduction saisonnière chez les ovins et les caprins (Use of melatonin implants and photoperiodic treatments to control seasonal reproduction in sheep and goats). *INRA Production Animale* **9**, 45–60.
- Chiappe de Cingalani GE, Goers JW, Giannotti M & Caldiz CI (1996) Comparative effects of insulin and isoproterenol on lipoprotein lipase in rat adipose cells. *American Journal of Physiology* **270**, C1461–C1467.
- Chilliard Y (1987) Revue bibliographique: Variations quantitatives et métabolisme des lipides dans les tissus adipeux et le foie au cours du cycle gestation-lactation. 2. Chez la brebis et la vache (Quantitative variations and metabolism of lipids in adipose tissue and liver during gestation-lactation cycle. 2. In the ewe and cow). *Reproduction Nutrition Development* **27**, 327–398.
- Chilliard Y & Bocquier F (2000) Direct effects of photoperiod on lipid metabolism, leptin synthesis and milk secretion in adult sheep. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 205–223 [PB Cronjé, editor]. Wallingford: CABI Publishing.
- Chilliard Y, Gagliostro G, Fléchet J, Lefaivre J & Sebastian I (1991) Duodenal rapeseed oil infusion in early and midlactation cows. 5. Milk fatty acids and adipose tissue lipogenic activities. *Journal of Dairy Science* **74**, 1844–1854.
- Chilliard Y, Sauviant D, Bas P, Pascal G & Morand-Fehr P (1981) Importance relative et activités métaboliques des différents tissus adipeux de la chèvre laitière. (Relative importance and metabolic activities of various adipose tissues in dairy goats). In *Nutrition et Systèmes d'Alimentation de la Chèvre*, vol. 1, pp. 80–89 [INRA-ITOVIC, editor]. Paris: INRA.
- Chilliard Y, Sauviant D & Morand-Fehr P (1979) Goat mammary, adipose and milk lipoprotein lipases. *Annales de Recherche Vétérinaire* **10**, 401–403.
- Cruz ML & Williamson DH (1992) Refeeding meal-fed rats increases lipoprotein lipase activity and deposition of dietary [¹⁴C] lipid in white adipose tissue and decreases oxidation to ¹⁴CO₂. *Biochemical Journal* **285**, 773–778.
- DiMarco NM, Beitz DC & Whitehurst GB (1981) Effect of fasting on free fatty acid, glycerol and cholesterol concentrations in blood plasma and lipoprotein lipase activity in adipose tissue of cattle. *Journal of Animal Science* **52**, 75–82.
- Eckel RH (1987) Adipose tissue lipoprotein lipase. In *Lipoprotein Lipase*, Chapter 4, pp. 79–132 [J Borensztajn, editor]. Chicago, IL: Evener Publishing.
- Emane MN, Delouis C, Kelly PA & Djiane J (1986) Evolution of prolactin and placental lactogen receptors in ewes during pregnancy and lactation. *Endocrinology* **118**, 695–700.
- English DE, Russel SM, Kartz LS & Nicoll CS (1990) Evidence for a role of the liver in the mammatrophic action of prolactin. *Endocrinology* **126**, 2252–2256.
- Faulconnier Y, Bonnet M, Fléchet J, Bocquier F & Chilliard Y (1999) Nutritional regulation of lipoprotein lipase activity in bovine adipose tissues and muscles. *Proceedings of the Nutrition Society* **58**, 108A.
- Faulconnier Y, Thévenet M, Fléchet J & Chilliard Y (1994) Lipoprotein lipase and metabolic activities in incubated bovine adipose tissue explants: Effects of insulin, dexamethasone, and fetal bovine serum. *Journal of Animal Science* **72**, 184–191.
- Hocquette JF, Graulet B & Olivecrona T (1998) Lipoprotein lipase activity and mRNA levels in bovine tissues. *Comparative Biochemistry and Physiology Part B* **121**, 201–212.

- Ingle DL, Bauman DE, Mellenberger RW & Johnson DE (1973) Lipogenesis in the ruminant: effect of fasting and refeeding on fatty acid synthesis and enzymatic activity of sheep adipose tissue. *Journal of Nutrition* **103**, 1479–1488.
- Institut National de la Recherche Agronomique (1989) Recommended allowances and feed tables. In *Ruminants Nutrition* [R Jarrige, editors]. Paris: John Libbey Eurotext.
- Ladu MJ, Kapsas H & Palmer WK (1991) Regulation of lipoprotein lipase in adipose and muscle tissues during fasting. *American Journal of Physiology* **260**, R953–R959.
- Larsen TS, Nilsson NÖ & Blix AS (1985a) Seasonal changes in lipogenesis in isolated adipocytes from Svalbard and Norwegian reindeer. *Acta Physiologica Scandinavica* **123**, 53–59.
- Larsen TS, Nilsson NÖ & Schytte Blix A (1985b) Seasonal changes in lipogenesis and lipolysis in isolated adipocytes from Svalbard and Norwegian reindeer. *Acta Physiologica Scandinavica* **123**, 97–104.
- Le Gouic S, Atgé C, Viguerie-Bascands N, Haoun N, Larrouy D, Ambid L, Rimbault S, Ricquier DP, Gardiola-Lemaitre B, Pénicaud L & Casteilla L (1997) Characterisation of a melatonin binding site in Siberian hamster brown adipose tissue. *European Journal of Pharmacology* **339**, 271–278.
- Lincoln GA & Richardson M (1998) Photo-endocrine control of seasonal cycles in body weight, pelage growth and reproduction: lessons from the HPD sheep model. *Comparative Biochemistry and Physiology* **119C**, 283–294.
- Meier AH & Cincotta AH (1996) Circadian rhythms regulate the expression of the thrifty genotype/phenotype. *Diabetes Reviews* **4**, 464–487.
- Mercer JG (1998) Regulation of appetite and body weight in seasonal mammals. *Comparative Biochemistry and Physiology* **119C**, 295–303.
- Ng TB & Wong CM (1986) Effects of pineal indoles and arginine vasotocin on lipolysis and lipogenesis in isolated adipocytes. *Journal of Pineal Research* **3**, 55–56.
- Ong JM, Simsolo RB, Saghizadeh M, Pauer A & Kern PA (1994) Expression of lipoprotein lipase in rat muscle: regulation by feeding and hypothyroidism. *Journal of Lipid Research* **35**, 1542–1551.
- Ortavant R, Bocquier F, Pelletier JP, Ravault JP, Thimonier J & Volland-Nail P (1988) Seasonality of reproduction in sheep and its control by photoperiod. *Australian Journal of Biology Science* **41**, 69–85.
- Pang CS, Brown GM, Tang PL, Cheng KM & Pang SF (1993) 2-[125I]iodomelatonin binding sites in the lung and heart: a link between the photoperiodic signal, melatonin, and the cardiopulmonary system. *Biological Signals* **2**, 228–236.
- Quig DW, Layman DK, Bechtel PJ & Hackler LR (1983) The influence of starvation and refeeding on the lipoprotein lipase activity of skeletal muscle and adipose tissue of lean and obese Zucker rats. *Journal of Nutrition* **113**, 1150–1156.
- Reimers E, Ringberg T & Sorumgaard R (1982) Body composition of Svalbard reindeer. *Canadian Journal of Zoology* **60**, 1812–1821.
- Renz M, Tomlinson E, Hultgren B, Levin N, Gu Q, Shimkets RA, Lewin DA & Stewart TA (2000) Quantitative expression analysis of genes regulated by both obesity and leptin reveals a regulatory loop between leptin and pituitary-derived ACTH. *Journal of Biological Chemistry* **275**, 10429–10436.
- Sugden MC, Holness MJ & Howard RM (1993) Changes in lipoprotein lipase activities in adipose tissue, heart and skeletal muscle during continuous or interrupted feeding. *Biochemical Journal* **292**, 113–119.
- Vernon RG (1989) Endocrine control of metabolic adaptation during lactation. *Proceedings of the Nutrition Society* **48**, 23–32.
- Vernon RG, Clegg RA & Flint DJ (1986) Adipose tissue metabolism in sheep: response to season and its modulation by reproductive state. *Hormone Metabolism Research* **18**, 308–312.
- Vernon RG & Taylor E (1986) Enzymes of adenosine metabolism of sheep adipose tissue: changes in activity with season, pregnancy and lactation. *Hormone and Metabolic Research* **18**, 369–373.
- Wade N & Bartness TJ (1984) Effects of photoperiod and gonadectomy on food intake, body weight, and body composition in Siberian hamsters. *American Journal of Physiology* **246**, R26–R30.
- Wolden-Hanson T, Mitton DR, McCants L, Yellon SM, Wilkinson CW, Matsumoto AM & Rasmussen DD (2000) Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology* **141**, 487–497.
- Youngstrom TG & Bartness TJ (1995) Catecholaminergic innervation of white adipose tissue in Siberian hamsters. *American Physiological Society* **268**, R744–R751.