Liver catalase in Southdown sheep selected for high and low backfat depth

S.W. PETERSON AND R.W. PURCHAS

Animal Science Department, Massey University, Palmerston North

ABSTRACT

Because the enzyme catalase is involved in the peroxisomal pathway of fatty acid oxidation in the liver, it is possible that higher levels of catalase activity may be associated with higher levels of fatness. This was investigated in the high and low backfat Southdown sheep selection lines at Massey University.

Liver biopsies were taken from 13 high backfat (HBD) and 14 low backfat (LBD) ewe hoggets with mean liveweight-adjusted ultrasonic backfat depths (LABF, expressed as percentage deviations) of +42% and -20% respectively. The HBD group had significantly higher ($P < 0.05$) liver catalase activity (1944 ± 109 K/g) than the LBD group (1649 ± 86 K/g). In a second trial, liver samples from 5 HBD rams showed significantly ($P < 0.05$) higher mean catalase activity (4708 ± 449 K/g) than samples from 5 LBD rams (3087 ± 434 K/g). Mean ultrasonic fat depth C measurements of progeny-groups of these rams (293 lambs in total) differed significantly ($P < 0.01$) between sires and the regression of progeny-group mean fat depth C on sire liver catalase activity was significant ($P = 0.067$; $r = 0.60$). Mean progeny-group carcass GR differed significantly between sires ($P < 0.01$) and the regression of mean progeny-group GR on sire catalase activity was significant ($P < 0.05$; $r = 0.64$). Lower levels of liver catalase activity may be a useful marker for identifying leaner genotypes.

Keywords Catalase; sheep liver; fatty acid metabolism; fatness; progeny test

INTRODUCTION

Proxisomes are a type of microbody characterised by the presence of an enzyme generating hydrogen peroxide (H_2O_2). Since H_2O_2 is a strong oxidising agent, peroxisomes also characteristically contain the enzyme catalase which reduces H_2O_2 to water.

Peroxisomes in different organisms and tissues contain various overlapping sets of enzymes (more than 40 identified) but so far no peroxisome has been found that has all the enzymes. Despite the wide functional diversity of peroxisomes, the metabolic function always present is the association of H_2O_2-producing oxidases with catalase. Substrates of this oxidase activity include amino acids, hydroxy acids, fatty acids, alcohols, amines and a purine, but not NADH. No energy retrieval mechanism has been found to be coupled to peroxisomal oxidations (de Duve, 1983). Hryb (1981) postulated that part of the energy of peroxisomal oxidation is conserved in the form of heat, and is used in the heating of the cell (chemical thermiogenesis). Liver peroxisomes have been shown to be a major site for the β-oxidation of long chain fatty acids in rats (Lazarow, 1978). Clofibrate (a drug used to lower blood fats) causes proliferation of peroxisomes in rat liver and induces peroxisomal β-oxidation (Osmundsen, 1982, de Duve, 1983). Peroxisomal β-oxidation can also be induced by high fat diets (Osmundsen, 1982; Neat *et al.* 1981) or by exposure to cold (de Duve, 1983); two circumstances in which enhanced fatty acid oxidation would be expected.

In contrast with mitochondrial β-oxidation the first enzyme of the peroxisomal sequence donates electrons directly to molecular oxygen thereby producing H_2O_2 (Mannaerts and Debeer, 1982). Peroxisomes are not capable of degrading a fatty acid completely to 2C acetyl units, but function as a chain shortening system that stops at C6 (Neat *et al.*, 1981; Mannaerts and Debeer 1982). Furthermore mitochondria are slow at oxidising some very long chain fatty acids (Mannaerts and Debeer, 1982) and peroxisomes can rapidly oxidise some trans-fatty acids which are slowly metabolised by mitochondria (Neat *et al.*, 1981; Osmundsen, 1982).

These findings suggest a role for peroxisomal β-oxidation in supplementing mitochondrial β-oxidation when the liver has a high load of fatty acids, especially when there is an appreciable

proportion of fatty acids that are poorly oxidised by mitochondrial β-oxidation. Since the primary objective is not energy generation, but shortening fatty acids to metabolically desirable lengths, it has been suggested (Neat *et al.*, 1981; Osmundsen, 1982) that there are advantages in not having the process linked to oxidative phosphorylation.

The nature of the peroxisome appears to lend itself to a role in the control of blood lipid concentration and fatty acid oxidation and so it may be associated with differences in fatness. The objective of the current study was to establish whether there were any differences in peroxisomal metabolism between the high backfat and low backfat lines of Southdown sheep at Massey University.

MATERIALS AMD METHODS

The Southdown sheep used in the two trials were from the Massey University backfat selection lines (Purchas *et al.*, 1982) but were not random samples from the two lines, as an initial culling on the basis of weight-corrected backfat depth and liveweight had taken place at an age of approximately nine months. The sheep used in these experiments were a random selection from the approximately 20 remaining animals per line.

The first trial involved 13 high backfat (HBD) and 14 low backafat (LBD) ewe hoggets (11 months of age). The hoggets were removed from pasture and penned overnight indoors. They were anaesthetised with pentabarbitone sodium (approx 0.9 cm^3 i.v.) before liver biopsies were taken by a veterinarian. Liver samples were stored in plastic vials at -12°C within 3 hours of collection.

The second trial involved 5 HBD and 5 LBD two-tooth rams which were single-sire mated to groups of about 40 mixed age Romney ewes (403 in total) randomly allocated to sire goup within age class.Rams were slaughtered 4-6 weeks after the end of mating, their livers collected onto ice and frozen at -12°C. Samples were later taken from the middle of the left lobe. All samples were analysed for catalase activity in the laboratory of Dr R. Munday at Ruakura using the method of Aebi (1974), in which the disappearance of H_2O_2 is followed spectrophoto-metrically. Samples were analysed in triplicate and results expressed in units of K/g wet weight.

Progeny of the 10 rams were slaughtered and carcass measurements made on about 30 lambs per sire group (293 lambs in total). Fat depth C (both sides) was assessed ultrasonically prior to transport of lambs to the abbatoir. Measurements of GR were made within 30 minutes of slaughter (1 side only). Least squares means (LSM) of GR and fat depth C, adjusted for sex, birthrank and weight, were calculated for each progeny-group.

RESULTS

Liver samples from HBD ewes had significantly higher mean catalase activity than the LBD group (Table 1). The mean backfat depth of the HBD group (5.9 ± 0.3mm) and that LBD group (3.3 ± 0.25mm) differed significantly ($P < 0.001$!). Mean liveweights of the HBD and LBD groups did not differ significantly after selection (May) or prior to biopsy (July) (Table 1). The regression of ewe liver catalase activity on LABF (Table 3) was not significant.

Liver samples from the HBD rams showed significantly higher mean catalase activity than the LBD group (Table 2). The regression of liver

TABLE 1 Mean liver catalase activity, live weights and LABF for the two backfat selection lines of ewe hoggets.

		Ewe selection line		Line
		HBD	LBD	effect
n		13	14	
Ewe LABF	(%dev ± SE)	42 ± 7	20 ± 6	***
Liver catalase activity	(K/g ± SE)	1944 ± 109	1649 ± 86	*
Live weight (May)	(kg ± SE)	34.0 ± 0.5	34.5 ± 0.8	NS
Live weight (July)	(kg ± SE)	35.6 ± 0.8	34.2 ± 0.9	NS

TABLE 2 Mean liver catalase activity and LABF for the two backfat selection lines of ram hoggets and mean values for progeny-group fat depth C and GR measurement.

		Sire selection line HBD		LBD		Line effect
n		5		5		
Sire LABF	(%dev ± SE)	45.6	± 17.2	-28.1	± 7.5	**
Sire catalase activity	(K/g ± SE)	4708	± 449	3087	± 434	*
Progeny group fat-depth C	(mm ± SE)	2.51	± 0.07	2.08	± 0.07	**
Progeny group GR	(mm ± SE)	6.61	± 0.15	6.37	± 0.47	NS

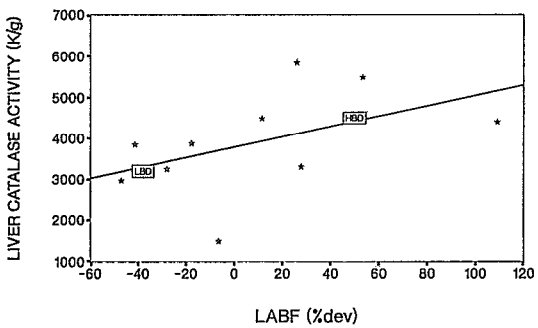

FIG. 1 Liver catalase activity in 5 high backfat depth (HBD) rams (closed stars) and 5 low backfat depth (LBD) rams (open stars) in relation to liveweight-adjusted backfat depth (LABF) expressed as percent deviation.

catalase activity on LABF (Fig. 1) was not significant (Table 3). Ultrasonic fat depth C measurements on 293 progeny of these rams differed significantly between the 10 sires ($P<0.01$). Fig. 2 shows the mean progeny group fat depth C plotted against sire catalase activity. The regression of mean (LSM) progeny fat depth C on sire catalase activity was significant (Table 3). Mean progeny-group fat depth C differed significantly between the HBD and LBD groups (Table 2). Progeny carcass GR differed significantly between sires overall ($P<0.01$) and the regression fo progeny-group GR on sire

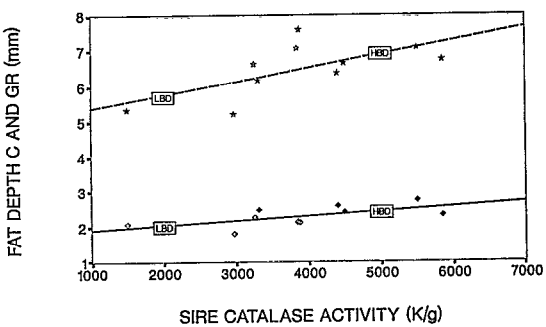

FIG. 2 Mean progeny-group fat depth C (——) and GR measurement (----) in relation to sire liver catalase activity. Values for high backfat depth (HBD) sheep are indicated by closed symbols and those for low backfat depth (LBD) sheep by open symbols.

TABLE 3 Regression relationships between liver catalase activity and measures of fatness both within animals and between sires and progeny.

Dependent variable	Independent variable	Regression coefficient±SE	Regression intercept±SE	Regression probability (*P*)	Correlation coeff.
Relationships within animals					
Ewe liver catalase (K)	LABF (%dev)	2.8 ± 1.9	1763 ± 74	>0.1	0.29
Ram liver catalase (K)	LABF (%dev)	12.7 ± 8.2	3787 ± 379	>0.1	0.48
Relationships between sires and progeny					
Progeny-group C (mm)	Sire liver catalase (K)	1.30×10^{-4} ± 0.61×10^{-4}	1.789 ± 0.250	0.067	0.60
Progeny-group GR (mm)	Sire liver catalase (K)	3.79×10^{-4} ± 1.61×10^{-4}	5.012 ± 0.656	0.046	0.64

catalase activity was significant (Table 3 and Fig.2).

However the man progeny-group GR values did not differ significantly (Table 2) between the HBD and LBD groups. It should be noted that the correlation coefficients in Table 3 may be inflated because the sheep were selected from the two genetic lines.

DISCUSSION

The role of peroxisomes in lipid metabolism is now well established (de Duve 1983; Lazarow, 1978) and there is substantial evidence that catalase is involved in lipid metabolism (Masters and Holmes, 1977). If liver peroxisomal activity (as measured by catalase activity) does make an important contribution to the extent to which blood lipids are oxidised (see introduction), then it might be expected that higher liver catalase activity would be associated with lower rates of fat deposition and leaner sheep.

The finding that HBD sheep in fact had higher catalase activity does not support this simplistic proposal. It is more likely that elevated catalase activity merely reflected higher fat turnover in the HBD sheep. Sidhu *et al* (1973) found that basal lipolysis increased with fatness in lamb adipose tissue and postulated that increased lipogenesis must compensate for this. Peterson (1984) reported that fasted HBD ewes exhibited quicker declines in respiratory exchange ratio than LBD ewes which is consistent with faster mobilisation of long chain fatty acids.

Liver peroxisomes are probably more important for specific functions such as shortening long chain fatty acids which mitochondria are not well equipped to handle, or in the provision of short chain fatty acids for specific purposes (e.g. pregnancy, lactation, growth) rather than for general control of overall blood lipid levels. Pregnant ewe hoggets sampled at the same time as the ewes in this study had significantly elevated liver catalase activity (SWPeterson, personal communication). Small *et al.* (1985) suggested a chain-shortening role for peroxisomes in the liver of pregnant and lactating rats as a means of supplying suitable substrates to the foetus and mammary gland.

Irrespective of whether catalase is causally related to fatness the results presented here suggest that lower liver catalase activity may be a useful marker for identifying lean genotypes of sheep, although further work is required to confirm these results which are based on relatively small numbers.

ACKNOWLEDGEMENTS

Bruce Farquharson for liver biopsies, Max Merrall for anaesthesia and Dr Rex Munday for the catalase assays. The late Dr Karl Jagusch provided the initial idea for this study.

REFERENCES

Aebi H. 1974. Catalase. *In* Methods of enzymatic analysis. 2nd Edition. *Ed* H.U.Bergmeyer. Academic Press New York and London. p 673-684.

de Duve. 1983. Microbodies in the living cell. *Scientific American* **248** (5): 52-62.

Hryb D.J. 1981. Peroxisomal respiration and energy conservation. *Febs letters* 128 (1): 1-4.

Lazarow P.B. 1978. Rat liver peroxisomes catalyse the β-oxidation of fatty acids. *Journal of biological chemistry* **253**:1522-1528.

Mannaerts G.P.; Debeer L.J. 1982. Mitochondrial and peroxisomal ·-oxidation of fatty acids in rat liver. *Annals of the New York Academy of Sciences* **386**: 30-39.

Masters C.J.; Holmes R.S. 1977. The metabolic roles of peroxisomes in mammalian tissues. *International journal of biochemistry* **8**: 549-553.

Neat C.E.;Thomassen M.S.;Osmundsen H. 1981. Effects of high-fat diets on hepatic fatty acid oxidation in the rat. *Biochemistry journal* **196**: 149- 159.

Osmundsen H. 1982. Peroxisomal β-oxidation of long fatty acids: effects of high fat diets. *Annals of the New York Academy of Sciences* **386**:13-29.

Peterson S.W. 1984. The breath-test for sheep: a possible means of identifying lean genotypes. *Proceeding of the New Zealand Society of Animal Production* **44**: 205-207.

Purchas R.W.; Rae A.L.; Barton R.A. 1982. Repeatability of weight-corrected ultrasonic fat-depth measurements made on ewes at intervals of one year. *New Zealand journal of agricultural research* **25**: 185-190.

Sidhu K.S.; Emery R.S.; Parr A.F.; Merkel R.A. 1973. Fat mobilising lipase in relation to fatness in lambs. *Journal of animal science* **36**:658-662.

Small G.M.; Burdett K.; Connock M.J. 1985. Changes in some peroxisomal enzyme activities during pregnancy and lactation in the rat. *Biochemical Society transactions* **13**: 872-873.