GLUCOSE METABOLISM IN FAT AND THIN ADULT SHEEP

M. A. McNIVEN[1]

Department of Animal Nutrition, Agricultural University of Norway, 1432 Aas-NLH, Norway. *Received 28 Oct. 1983, accepted 20 June 1984.*

McNIVEN, M. A. 1984. Glucose metabolism in fat and thin adult sheep. Can. J. Anim. Sci. **64**: 825–832.

The effect of body fatness on glucose metabolism was studied in six adult crossbred wethers divided into two groups weighing 90 kg (fat) or 55 kg (thin). Glucose turnover measurements were made using 6-^{3}H-glucose by single injection at four levels of metabolizable energy intake: high, 17 MJ/day; intermediate, 10 MJ/day; low, 6 MJ/day; and fasting for both thin and fat groups. The greatest group differences occurred at the high feeding level. Body pool size and glucose space were significantly lower ($P<0.001$) for the fat sheep and were reduced at fasting for both fatness levels. The mean irreversible loss rate was not affected by body fatness but was significantly affected ($P<0.001$) by feeding level. Glucose recycled as a proportion of total entry rate was significantly affected by body fatness and was dependent on a positive or negative energy balance. When in negative energy balance the sheep maintained blood glucose within the normal range by recycling up to four times more glucose than was utilized. It appears that rate of glucose synthesis is related to energy balance and metabolizable energy intake. A tendency toward insulin insensitivity in the fat sheep could be related to high levels of recycling, and a greater glucose drain or prolonged fast could be more serious with the decreased glucose pool size and space.

Key words: Glucose metabolism, ruminant, obese, energy balance

[Métabolisme du glucose chez des moutons adultes gras et maigres.]
Titre abrégé: Métabolisme du glucose chez des moutons gras et maigres.
Nous avons étudié les effets de l'état d'engraissement sur le métabolisme du glucose chez six mâles croisés castrés divisés en deux groupes: 90 kg (gras) et 55 kg (maigres). Nous avons mesuré le taux de renouvellement du glucose au moyen d'une injection unique de 6-^{3}H-glucose à quatre niveaux d'absorption de l'énergie métabolisable: élevé (17 MJ/j); intermédiaire (10 MJ/j); faible (6 MJ/j) et nul (jeûne) chez les sujets des deux groupes. Le régime à 17 MJ/j a provoqué la plus grande différence entre les deux groupes expérimentaux. L'importance du pool de glucose et le taux de glucose en pourcentage du poids total étaient significativement inférieurs ($P<0,001$) chez les moutons gras et ont par ailleurs subi une baisse lors du jeûne chez les sujets des deux groupes. Le taux moyen de perte irréversible ne change pas selon l'état d'engraissement mais il varie de façon significative ($P<0,001$) selon le niveau d'absorption d'énergie. Le taux de glucose recyclé sur la quantité totale absorbée variait significativement d'un groupe à l'autre et dépendait du bilan énergétique. Lorsque ce dernier était négatif, les moutons maintenaient leur taux de glucose sanguin à son niveau normal en recyclant jusqu'à quatre fois plus de glucose qu'ils n'en utilisaient. Nos résultats donnent à penser qu'il existe un lien entre le taux de synthèse du glucose d'une part, et le bilan énergétique et l'apport d'énergie métabolisable d'autre part. Une tendance, chez les moutons gras, à montrer une insensibilité à l'insuline pourrait être liée à des taux de recyclage

[1]Present address (M.A. McN.): Department of Animal Science, Macdonald College of McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec H9X 1C0.

élevés et une perte plus importante de glucose ou un jeûne prolongé pourraient avoir des conséquences plus graves chez des sujets dont le pool de glucose et le taux de glucose en pourcentage du poids sont réduits.

Mots clés: Métabolisme du glucose, ruminant, obèse, bilan énergétique

The metabolic effects of obesity have been studied extensively in monogastric animals (e.g. Schemmel 1980; Wangsness et al. 1981; McCracken and McNiven 1983) but little work has been done on the effects of body fatness in ruminants. High energy diets and group feeding have increased the prevalence of overfat cows with the resulting increased susceptibility to a variety of disorders (Morrow 1976).

Gluconeogenesis is of prime importance for ruminant metabolism since most of the carbohydrates ingested are fermented in the rumen and only small amounts of glucose are absorbed from normal diets. The supply of glucose precursors and the organs that synthesize glucose could be limiting factors for the animal's overall metabolism and even for its survival (Bergman 1973).

Abnormalities in glucose metabolism resulting from obesity are clearly documented in monogastrics (Lavau et al. 1979; Digirolamo 1981). It is interesting to note that before the isolation of insulin by Banting and Best in 1921, the principal method to prevent the ketoacidosis of diabetes was a starvation diet which lowered blood glucose and reduced depot fat stores to the point where there was little fat to mobilize (Ganong 1979).

The present study was undertaken in order to demonstrate the detrimental effects of excessive body fatness on glucose metabolism in adult sheep.

MATERIALS AND METHODS

Six adult, crossbred wethers initially weighing 60 kg were randomly allotted to two groups, fat and thin, and were fed at two levels for 4–5 mo to obtain body weights of about 90 and 55 kg, respectively. Balance and respiration trials, and glucose metabolism measurements were made on the two fatness groups on each of four levels of metabolizable energy (ME) intake: high, 17 MJ/day; intermediate, 10 MJ/day; low, 6 MJ/day; and fasting (Day 5 of fast). The intermediate level always preceded the fasting measurements.

The animals were accustomed to the metabolism cages. Rations, consisting of maize and high quality grass hay in equal proportions, were given in equal meals at 0730 and 1930 h and fresh water was available at all times. The animals received sufficient digestible protein, minerals and vitamins according to standards (National Academy of Sciences-National Research Council 1975). Methods and results of balance and respiration trials, and feed chemical analysis are reported elsewhere (McNiven 1984a). A model that included level of fatness, level of feeding and the interaction between the two as fixed effects was fitted to the data using General Linear Models (SAS Institute Inc. 1982).

Measurements of Glucose Metabolism

The sheep were administered 150 μCi of 6-^{3}H-glucose (specific activity 500 mCi/mmol; Amersham, England) in 10 mL saline (0.9% wt/vol NaCl) through an 18-gauge indwelling catheter inserted in the jugular vein, about 2 h after the morning feeding. Sixteen 7-mL blood samples were withdrawn over a period of 5 h beginning at 5 min after tracer injection. Blood samples were collected in heparinized tubes containing NaF (10–20 mg) to prevent glycolysis. The sampled blood was immediately chilled and centrifuged.

Plasma ^{3}H-glucose specific activities were determined on individual blood samples by the glucose pentaacetate method (Jones 1965). Preparation of glucose pentaacetate crystals was completed within 5 days of collection. Weighed amounts of pentaacetate crystals were dissolved in 10 mL scintillation liquid (toluene + 0.5% PPO + 0.05% dimethyl POPOP) and counted on a Packard Tri-Carb 4530 liquid scintillation spectrometer. Specific activity was expressed as nCi/mmol of plasma glucose by correcting for counting efficiency. Plasma glucose concentration was determined by reduction of ferricyanide and measured on a spectrophotometer (Technicon Autoanalyser).

Curves of ^{3}H-glucose specific radioactivity versus time were fit as the sum of two exponen-

tials using an iterative method to determine least-square estimates (SAS Institute Inc. 1982). Pool size, total entry rate, irreversible loss and recycling rates were calculated using techniques described by White et al. (1969). Glucose space was calculated by dividing the pool size of glucose by the corresponding plasma concentration and expressing the value as a percentage of body weight. The value for plasma concentration used in these calculations was the mean of 16 samples.

Glucose total entry rate represents the rate at which glucose from all sources enters and leaves the sampled compartment. Irreversible loss represents the rate at which a portion of the total entry rate leaves the sampled pool and never returns. Irreversible loss, therefore, represents the net quantity of glucose being utilized or which must be synthesized (gluconeogenesis) and (or) absorbed from the gut per unit time. It has also been referred to as glucose utilization rate, glucose entry rate and glucose turnover, depending largely on the method of calculation and isotope dilution technique used (Herbein et al. 1978).

Recycling, calculated by difference, represents the rate at which the remaining portion of the total entry rate leaves the sampled pool and returns. Total entry rate is, therefore, the sum of the irreversible loss rate and the recycling rate.

RESULTS

Table 1 shows the mean body weights, ME intakes and energy balances for the thin and fat sheep at four intake levels.

The glucose metabolism measurements for the thin and fat sheep are shown in Table 2. Body pool size of glucose and glucose space (percent of body weight) were significantly greater ($P<0.001$) for the thin sheep. Both parameters were reduced at fasting.

The mean total entry rate was higher for the fat sheep ($P<0.05$). Large differences between the fat and thin sheep on the high and intermediate levels caused the main effects interaction to be significant ($P<0.001$).

The mean values for irreversible loss rate for the fat and thin sheep were not significantly different. However, the individual means for the high level were significantly different and this caused the significant interaction ($P<0.05$) between main effects. The differences between feeding levels were highly significant ($P<0.01$) and the values were much lower at fasting for both fatness levels. When irreversible loss was expressed relative to metabolic body size, the mean value for the thin sheep was significantly greater ($P<0.01$) than for the fat sheep.

Table 3 shows the amount of glucose recycled and its relationship to total entry rate and irreversible loss rate. It did not appear that feeding level had a significant effect on recycling rate. However, when the proportions of glucose recycled are considered with the energy balance results a similarity appears. Although feed intakes were similar for the thin and fat sheep at each level of feed intake, energy balances were not. It appears that when energy balance became negative, the proportion of glucose that was recycled increased greatly. Expressed in a different way, the ratio of recycled glucose to glucose utilized (irreversibly lost) shows (Table 3) how the fat sheep, upon experiencing a negative energy balance (intermediate level), responded by increasing the recycling rate of glucose until almost four times more glucose was recycled than was utilized. The effect was similar, though not as large, for the thin sheep at fasting.

Correlation coefficients between energy balance, ME intake or body weight and glucose metabolism parameters are shown in Table 4. For each parameter, the correlation coefficients were higher for energy balance as compared with ME intake. All but one of the parameters were significantly correlated with energy balance while there were several parameters which were not significantly correlated with ME intake. Body weight was significantly correlated with glucose space ($P<0.001$). Glucose space is expressed as a percentage of body weight so the high correlation coefficient was expected.

DISCUSSION

Blood concentrations of glucose, free fatty acids, insulin, thyroxine and free thyroxine

Table 1. Body weight, metabolizable energy (ME) intake and energy balance for the thin and fat sheep

Feeding level	No. of animals		Body weight (kg)		ME intake (MJ/day)		Energy balance (MJ/day)	
	Thin	Fat	Thin	Fat	Thin	Fat	Thin	Fat
High	3	3	61(3.1)†	88(3.3)	16.8(1.0)	16.9(1.1)	4.3(0.8)	2.8(0.9)
Intermediate	3	3	59(3.3)	85(1.8)	10.2(0.2)	9.9(0.7)	0.5(0.3)	−1.7(0.5)
Low	3	3	53(4.0)	83(2.6)	6.7(0.2)	6.4(0.4)	−1.0(0.4)	−4.3(0.5)
Fast	3	3	55(2.6)	82(1.7)	0	0	−7.1(0.4)	−8.6(0.6)

†Standard deviation in parentheses.

Table 2. Glucose metabolism measurements for the thin and fat sheep at four levels of ME intake

Feeding level	Body pool size (mmol/kg$^{0.75}$)		Glucose space (% of body wt)		Total entry rate (mmol/min)		IRR† (mmol/min)		IRR† (μmol/kg$^{0.75}$/min)	
	Thin	Fat	Thin	Fat	Thin	Fat	Thin	Fat	Thin	Fat
High	2.8	1.8	26.1	17.3	2.01	1.18	1.14	0.72	52.6	25.0
Intermediate	2.2	1.5	21.0	12.6	0.97	2.44	0.59	0.70	27.8	25.1
Low	2.9	2.0	30.1	17.6	1.56	2.27	0.86	0.68	43.7	24.8
Fast	1.7	1.5	16.3	13.5	1.34	2.02	0.39	0.42	19.3	15.6
Least square	2.4	1.7	23.4	15.3	1.47	1.97	0.75	0.63	35.8	22.6
mean ± SE	±0.1	±0.1	±0.9	±0.9	±0.13	±0.13	±0.04	±0.04	±1.9	±1.9
Statistical significance										
Fatness level	***		***		*		NS		***	
Feeding level	***		***		NS		***		***	
Interaction	NS		NS		**		*		*	

†Irreversible loss rate.
*,**,***$P<0.05$, $P<0.01$, and $P<0.001$, respectively; NS = not significant.

Table 3. Glucose recycling and its relationship to total entry rate and irreversible loss rate

Feeding level	Recycling rate (mmol/min)		Recycling rate/total entry rate		Recycling rate/ irreversible loss rate	
	Thin	Fat	Thin	Fat	Thin	Fat
High	0.88	0.46	0.42	0.39	0.80	0.72
Intermediate	0.38	1.74	0.38	0.71	0.63	2.47
Low	0.56	1.59	0.35	0.68	0.55	2.35
Fast	0.95	1.60	0.67	0.79	2.69	3.79
Least square mean ± SE	0.69 ± 0.13	1.35 ± 0.12	0.45 ± 0.04	0.64 ± 0.03	1.17 ± 0.27	2.33 ± 0.26
Statistical significance						
Fatness level	**		**		**	
Feeding level	NS		**		**	
Interaction	*		NS		NS	

*,**,***$P<0.05$, $P<0.01$, and $P<0.001$, respectively; NS = not significant.

were determined five times during the day for the four levels of ME intake. The results are reported elsewhere (McNiven 1984b).

The present experiment confirms the statement of Steel and Leng (1973) that the rate of glucose synthesis in non-pregnant sheep is proportional to digestible energy intake. Herbein et al. (1978) suggested that daily energy intake might be a controlling factor in daily glucose turnover. They found a highly significant linear relationship between digestible energy intake and glucose turnover, despite differences in animal size, diet and physiological conditions (fasted versus fed, growing versus mature, lactating versus non-lactating). However, in the present experiment glucose turnover appeared to be better correlated to energy balance suggesting that the availability of glucose precursors absorbed from the digestive tract is not the only determining factor of glucose turnover. With regard to recycling rate, energy balance was more highly correlated than ME intake suggesting that body size can have a definite effect on the amount of glucose recycled insofar as it determines energy balance.

Differences in glucose metabolism measurements between the thin and fat sheep were greater when the sheep received feed while the differences became less or disappeared at fasting. Similar responses were observed by Cote et al. (1982) with obese pigs which showed no impaired glucose clearance during fasting but upon normal feeding, insulin resistance and impaired glucose tolerance became more apparent. It appears that mechanisms which are required to effect an increase in rate of glucose clearance in response to increased sub-

Table 4. Correlation coefficients (r) between energy balance, ME intake or body weight and glucose metabolism parameters ($n = 23$ or 24)

	Energy balance	ME intake	Body weight
Pool size	0.526**	0.500**	−0.024
Glucose space	0.493**	0.296	−0.642***
Total energy rate (TER)	−0.166	−0.066	0.395
Irreversible loss (IRR)	0.720***	0.672***	−0.129
IRR/$kg^{0.75}$	0.665***	0.535**	−0.447*
Recycling rate (RR)	−0.471*	−0.348	0.462*
RR/TER	−0.707***	−0.603**	0.365
RR/IRR	−0.735***	−0.650***	0.296

*,**,***$P<0.05$, $P<0.01$, and $P<0.001$, respectively.

strate concentration may be impaired in the obese animals.

Within each feeding level, the proportions of glucose recycled were very different for the thin and fat sheep. As soon as the fat sheep were in negative energy balance (−1.7 MJ/day) the proportion of glucose recycled increased from 39% to 71%. At fasting, the amount recycled was 79%. The thin sheep did not react as markedly and when first in negative energy balance (−1 MJ/day) the proportion recycled was only 35%. At fasting this value increased to 67%.

The increased proportion of glucose recycled in the total glucose entry rate is probably one compensatory method to spare body protein and maintain plasma glucose concentration within the normal range during negative energy balance. The fact that at fasting the thin sheep recycled almost three times more glucose than was utilized substantiates this statement. It appears that the fat sheep have an exaggerated response in recycling rate to the negative energy balance. This is probably related to a decreased insulin sensitivity of adipose tissue since when the feed intake was decreased from high to intermediate or low, blood insulin and glucose concentrations increased (McNiven 1984b). Steel and Leng (1973) did not find a significant increase in the recycling rate at fasting for pregnant and nonpregnant ewes, but they suggested that this may have been due to the low precision of estimation, and that recycling may have been increased at fasting.

It is important to clarify what recycling involves and the effect different isotopes can have on the amount of recycling.

Physical recycling or recirculation occurs when the label leaves the sampled pool and returns in unchanged glucose molecules. Chemical recycling is the return of the label in glucose molecules resynthesized from labelled glucose metabolites.

Brockman et al. (1975) using 6-^{3}H-glucose and U-^{14}C-glucose determined the extent to which ^{14}C was recycled when determining rates of glucose production in sheep. Such recycling of label would underestimate the rate of glucose production. They found that recycling (i.e. chemical recycling) was low and was not influenced by fasting for 3 days. In the present experiment using 6-^{3}H-glucose, the results represent mostly physical recycling and minimal chemical recycling and showed a definite effect of decreased feed intake and fasting on recycling rate. Judson and Leng (1972) compared various glucose labels for estimating total entry rate and resynthesis of glucose in sheep and concluded that 2-^{3}H-glucose and U-^{14}C-glucose were the most useful for studies of glucose metabolism, particularly for estimating the extent of resynthesis. Young (1977) proposed that 6-^{3}H-glucose was the best isotope to use for most purposes and suggested that this was the unofficial consensus of the Tracer Methodology Group (Bergman et al. 1974).

The use of single injection measurements is not recommended for use in estimating 24-h glucose entry rates when the feeding interval is 12 or 24 h (Young 1977). The absolute values in the present experiment should thus not be extrapolated to be representative of a 24-h steady-state period. On the other hand, the comparison of the results from the fat and thin sheep is valid because identical feeding interval and amounts of feed ensured that the non-steady state conditions were similar for both groups during measurements.

In the peripheral catabolism of 6-^{3}H-glucose to glycerol, tritium remains bound to carbon (Judson and Leng 1972). Differences in possible cycling of glucose carbon via the body glycerol pool could contribute to differences in irreversible loss from the glucose pool (Cote et al. 1982). Fat sheep appeared to have a greater degree of fat mobilization at fasting, as suggested by significantly higher levels of plasma free fatty acids than thin sheep (McNiven 1984b). In fasting obese men, 79% of new glucose was derived from glycerol, while in thin men, the corresponding value was 38% (Bortz et al. 1972). A greater dependence on glycerol turnover to supply glucose in the fat

sheep could perhaps have brought about the increase in fat mobilization and the differences in glucose turnover.

Cote et al. (1982) found similar differences in pool size, glucose space and irreversible loss rate of glucose when they compared lean and genetically obese pigs. They suggested that differences in glucose metabolism between fat and thin animals could be a result of differences in insulin sensitivity, fatty acid and amino acid turnover rates, or glucagon or glucocorticoid status.

In the present experiment, both the thin and the fat sheep at fasting were able to maintain normal blood glucose concentrations via gluconeogenesis, recycling and glucose-sparing mechanisms, in the absence of a glucose drain for production purposes. However, the reduced pool size of glucose and glucose space in the fat sheep show that the body's reserves of glucose were low and the normal blood glucose levels during fasting would probably not have been maintained much longer.

The tendency toward insulin resistance and excessive fat mobilization in the fat sheep (McNiven 1984b) and an excessive compensation in glucose metabolism during negative energy balance suggest that more serious effects of alterations in glucose metabolism were forthcoming. Bergman (1973) suggested that in ketosis in cattle and pregnancy toxemia in sheep following a negative energy balance and reduction of glucose in the blood and liver, the animal appeared to "overshoot" in the direction of excessive fat mobilization with its resultant ketosis.

The fat sheep in the present experiment did not exhibit many of the symptoms common to ruminants suffering from excessive fatness. Anorexia and an increased fasting heat production have been reported in extremely fat sheep (Graham 1969). Adipocytes reach a critical size after which they become less active. It is thought that the amount of water per cell becomes limiting and that the function of cytoplasmic enzymes using water-soluble substrates may be impaired (Hood and Allen 1975). The decrease in glucose space in the fat sheep is indicative of a decrease in total body water although no pathological symptoms were observed.

In conclusion, an excessive amount of fat in the body of ruminants can lead to alterations in glucose metabolism which, in turn, may result in an increased susceptibility to metabolic disorders such as ketosis, Fat Cow Syndrome and pregnancy toxemia in some animals, particularly those in negative energy balance or with an increased glucose drain.

ACKNOWLEDGMENTS

This study was supported by the Norwegian Agricultural Research Council.

BERGMAN, E. N. 1973. Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Vet. **63**: 341–382.

BERGMAN, E. N., BROCKMAN, R. P. and KAUFMAN, C. F. 1974. Glucose metabolism in ruminants: comparison of whole-body turnover with production by gut, liver and kidneys. Fed. Proc. **33**: 1849–1854.

BORTZ, W. M., PAUL, P., HAFF, A. C. and HOLMES, W. L. 1972. Glycerol turnover and oxidation in man. J. Clin. Invest. **51**: 1537–1546.

BROCKMAN, R. P., BERGMAN, E. N., POLLAK, W. L. and BRONDUM, J. 1975. Studies of glucose production in sheep using (6-^{3}H) glucose and (U-^{14}C) glucose. Can. J. Physiol. Pharmacol. **53**: 1186–1189.

COTE, P. J., WANGSNESS, P. J., VARELA-ALVAREZ, H., GRIEL, L. C. Jr., and KAVANAUGH, J. F. 1982. Glucose turnover in fast-growing, lean and in slow-growing obese swine. J. Anim. Sci. **54**: 89–94.

DIGIROLAMO, M. 1981. Effects of variable glucose and fat-cell concentration on glucose metabolism and insulin responsiveness by adipocytes of different sizes. Int. J. Obesity **5**: 671–677.

GANONG, W. F. 1979. Review of medical physiology. Lange Medical Publications, Los Altos, Calif.

GRAHAM, N. McC. 1969. The influence of body weight (fatness) on the energetic efficiency of adult sheep. Aust. J. Agric. Res. **20**: 375–385.

HERBEIN, J. H., VAN MAANEN, R. W.,

McGILLIARD, A. D. and YOUNG, J. W. 1978. Rumen propionate and blood glucose kinetics in growing cattle fed isoenergetic diets. J. Nutr. **108**: 994–1001.

HOOD, R. L. and ALLEN, C. E. 1975. Bovine lipogenesis: effects of anatomical location, breed and adipose cell size. Int. J. Biochem. **6**: 121–131.

JONES, G. B. 1965. Determination of the specific activity of labelled blood glucose by liquid scintillation using glucose pentaacetate. Anal. Biochem. **12**: 249–258.

JUDSON, G. J. and LENG, R. A. 1972. Estimation of the entry rate and resynthesis of glucose in sheep using glucoses uniformly labelled with ^{14}C and variously labelled with ^{3}H. Aust. J. Biol. Sci. **25**: 1313–1332.

LAVAU, M., FRIED, S. K., SUSINI, C. and FREYCHET, P. 1979. Mechanism of insulin resistance in adipocytes of rats fed a high-fat diet. J. Lipid Res. **20**: 8–16.

McCRACKEN, K. J. and McNIVEN, M. A. 1983. Effects of overfeeding by gastric intubation on body composition of adult female rats and on heat production during feeding and fasting. Br. J. Nutr. **49**: 193–202.

McNIVEN, M. A. 1984a. The effect of body fatness on energetic efficiency and fasting heat production in adult sheep. Br. J. Nutr. **51**: 297–304.

McNIVEN, M. A. 1984b. Effect of body fatness on blood metabolites and insulin insensitivity in adult sheep. Can. J. Anim. Sci. **64**: 1049–1053.

MORROW, D. A. 1976. Fat cow syndrome. J. Dairy Sci. **59**: 1625–1629.

NATIONAL ACADEMY OF SCIENCE-NATIONAL RESEARCH COUNCIL. 1975. Nutrient requirements of domestic animals. No. 5. Nutrient requirements of sheep. 5th ed. NAS-NRC, Washington, D.C.

SAS INSTITUTE INC. 1982. SAS user's guide: Statistics. 1982 ed. SAS Institute Inc., Cary, N.C.

SCHEMMEL, R. 1980. Nutrition, physiology, and obesity. CRC Press, Inc., Boca Raton, Fla.

STEEL, J. W. and LENG, R. A. 1973. Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. Br. J. Nutr. **30**: 451–473.

WANGSNESS, P. J., ACKER, W. A., BURDETTE, J. H., KRABILL, L. F. and VASILATOS, R. 1981. Effect of fasting on hormones and metabolites in plasma of fast-growing, lean and slow-growing obese pigs. J. Anim. Sci. **52**: 69–74.

WHITE, R. G., STEEL, J. W., LENG, R. A. and LUICK, J. R. 1969. Evaluation of three isotope-dilution techniques for studying the kinetics of glucose metabolism in sheep. Biochem. J. **114** : 203–214.

YOUNG, J. W. 1977. Gluconeogenesis in cattle: significance and methodology. J. Dairy Sci. **60**: 1–15.