COMPARATIVE ASPECTS OF FUEL SUPPLY DURING EXERCISE

D.W. PETHICK

Summary

In this paper evidence is put forward to suggest that sheep are well adapted to maintain a supply of carbohydrate for contracting muscle when compared to other mammals such as the human athlete. This is achieved by a remarkable ability to maintain or even elevate glucose concentration in the blood through "feed forward" control of glucose metabolism and by high rates of gluconeogenesis. Glycogen, whether hepatic or muscle, is also readily utilised at work rates above the anaerobic threshold. The ability to replete glycogen is substantial in sheep which are adapted to exercise and is not greatly lower than the rates found in nonruminant animals. Aspects of long chain fatty acid (NEFA) metabolism are also discussed. An interesting finding in sheep is the partial oxidation of NEFA to acetate during sustained exercise at above the anaerobic threshold. This represents a mechanism to increase the power of fat oxidation and deserves attention in other species.

I. INTRODUCTION

In the simplest sense, exercise results in a greater requirement for nutrients to fuel the increased metabolic rate of contracting muscle. The balance and metabolic destination of these nutrients is determined principally by the work rate but diet and genetic potential also play a role. The major tissues which adapt to maintain nutrient balance are the gastrointestinal tract, liver, adipose tissue and skeletal muscle. This paper will discuss the role played by these tissues to maintain nutrient balance during extended exercise. The sheep will be the primary example with some reference to other species, particularly the human athlete.

Table 1. Power and Acceleration time of different energy sources

Energy Source	Maximum relative Power (%)	Acceleration time (secs)
Anaerobic:		
ATP	100	<1
Phosphocreatine	77	<1
Carbohydrate → Lactate	46	<5
Aerobic:		
Carbohydrate → $CO_2 + H_2O$	24	180
Fatty acid → $CO_2 + H_2O$	13	1800

School of Veterinary Studies, Murdoch University, Murdoch WA Australia 6150

II. PRINCIPLES OF FUEL USE

Several substrates can be utilised to form ATP for muscle contraction. However, for each substrate there is a limit on the rate of ATP production per unit time (power) and the time taken to reach maximal power (acceleration time). Table 1 summarises this information (Sahlin 1986).

(a) Maximum power of fatty acid oxidation

Three important break points in metabolism have been recognised and defined (Sahlin 1986). The first break point is the maximum power of fatty acid oxidation and corresponds to about 30-50% VO_2 max in the human athlete. This threshold is determined by such things as the concentration of nonesterified fatty acids (NEFA) in blood, the rate of blood flow and so O_2 delivery to muscle, the extent of intramuscular lipid utilisation and the mitochondrial density of the muscle cells. It should be emphasised that exercise below the anaerobic threshold will not necessarily be fueled by fat alone because it can take many minutes for NEFA concentration to increase in the plasma so as they can be utilised as a fuel. However very long term exercise (i.e. several hours) at below the anaerobic threshold will ultimately be fueled by fat.

(b) Anaerobic threshold

The next break point occurs when the anaerobic utilisation of carbohydrate becomes important. It is defined as a significant but stable increase in blood lactate concentration, and it occurs at 50-65% VO_2max (human studies) depending on genetic potential and state of training. There is controversy as to the initial cause of lactate accumulation and so some prefer to call this threshold the "onset of blood lactate". The most significant aspect of this threshold is the initiation of rapid glycogen utilisation and pronounced changes in hormone secretion, particularly the catecholamines. Thus at exercise above the anaerobic threshold the concentration of lactic acid and the catecholamines increase exponentially as work rate increases (Mazzeo and Marshall,1989). Exercise above the anaerobic threshold is potentially limited by the availability of carbohydrate reserves (glycogen).

(c) Phosphocreatine threshold

The final break point occurs at very high rates of exercise when phosphocreatine concentration becomes depleted within the muscle cell and corresponds to very high and continually increasing concentrations of lactate in the blood.

III. CARBOHYDRATE METABOLISM

(a) Feed forward control

In all species exercise prompts an acute increase in glucose turnover but the response of blood glucose is quiet variable. More recently the factors influencing glucose secretion by the liver and the resulting blood glucose concentration have been better understood. The level of nutrition is important, with fasting invariably attenuating any tendency for hyperglycemia particularly in nonruminants (Sonne et al. 1987). An even more important determinant of glucose metabolism is work rate. Below the anaerobic threshold euglycemia is maintained despite up to a two fold increase in glucose entry rate. However as the rate of exercise increases glucose production by the liver and the degree of hyperglycemia increase markedly (Pethick et al. 1991). This response suggests that at the lower levels of exercise there is normal feedback control of hepatic glucose

production such that increases closely match the extra requirements of exercise. At the higher work rates it is suggested that feedforward control operates (Vissing et al 1988; Kjaer et al. 1987). This implies an exaggerated sympathoadrenal response resulting in an increased glucose output by liver that is not readily influenced by normal feedback signals.

The tendency for feedforward control is probably species dependent although no controlled studies are available to make close comparisons. The sheep clearly shows a very large response and the human athlete a much smaller one. Thus even in the underfed pregnant ewe (fed 0.3 x maintenance), a state typified by hypoglycemia due to a large fetal requirement for glucose, metabolism responds dramatically to exercise. Walking at above the anaerobic threshold elicits a 2.4 fold increase in the glucose entry rate, a near 4 fold increase in glucose uptake by maternal tissues and a 1.7 fold increase in blood glucose concentration (Leury et al. 1990). In contrast exercise in pregnant women elicits hypoglycemia or euglycemia (Lotgering et al. 1985). This reflects the central role played by glucose as a fuel for exercise even in the sheep where many believed that glucose metabolism might be suppressed due to a lack of glucose absorption.

A probable benefit of hyperglycemia would be to facilitate concentration dependent glucose uptake by working muscle, although the effect would be indiscriminate (Pethick et al. 1991). In support of this Meszaros et al.(1989) showed that hyperglycemia as a result of catecholamine infusion into rats dramatically increased glucose uptake in most tissues except brain.

(b) Sustained exercise

Data from experiments performed in this laboratory (Harman 1991, Harman and Pethick 1987, Pethick and Harman 1989) form the basis of the following discussion. The experiments involved studying merino ewes at rest or while walking at 4.5km h^{-1} on 0^{o} incline (estimated as 30%VO_2max) for 4 hours or on 9^{o} incline for 2h (estimated as 60%VO_2max), the latter work rate resulted in near exhaustion. Whole animal and regional tissue metabolism were studied utilising the principles of isotope dilution and arteriovenous difference. All animals were fed to maintenance a diet of ground and pelleted lucerne fed semi continuously.

At rest, skeletal muscle of sheep uses considerable amounts of blood glucose, sufficient to account for half of the energy metabolism. As the level of exercise increases, the liver releases additional glucose which in turn is utilised by the exercising muscle. Table 2 shows data to support this notion. Importantly the rate of glucose release by liver and the uptake by muscle remained sustained and matched throughout the exercise period, resulting in no tendency for hypoglycemia.

Similar research using human subjects in the postabsorptive state exercised on a bicycle ergometer at 30% and 58% VO_2max has been performed by Ahlborg et al.(1974) and Ahlborg and Felig (1982). Exercise, like in the sheep, prompted an increase in the output of glucose by the liver which was matched by an increase in the uptake by muscle. The contribution of glucose to oxidative metabolism in muscle (assuming complete oxidation) during exercise was similar at 26-36%. A striking difference was the tendency for hypoglycemia. Exercise at 30% VO_2max resulted in a mild hypoglycemia while at 58% VO_2max a significant 37% decline in glucose concentration occurred after 3h of exercise. The major reason for this hypoglycemia was reduced hepatic output of glucose (down by 50% after 3h of exercise at 58% VO_2max) in the face of sustained muscle uptake.

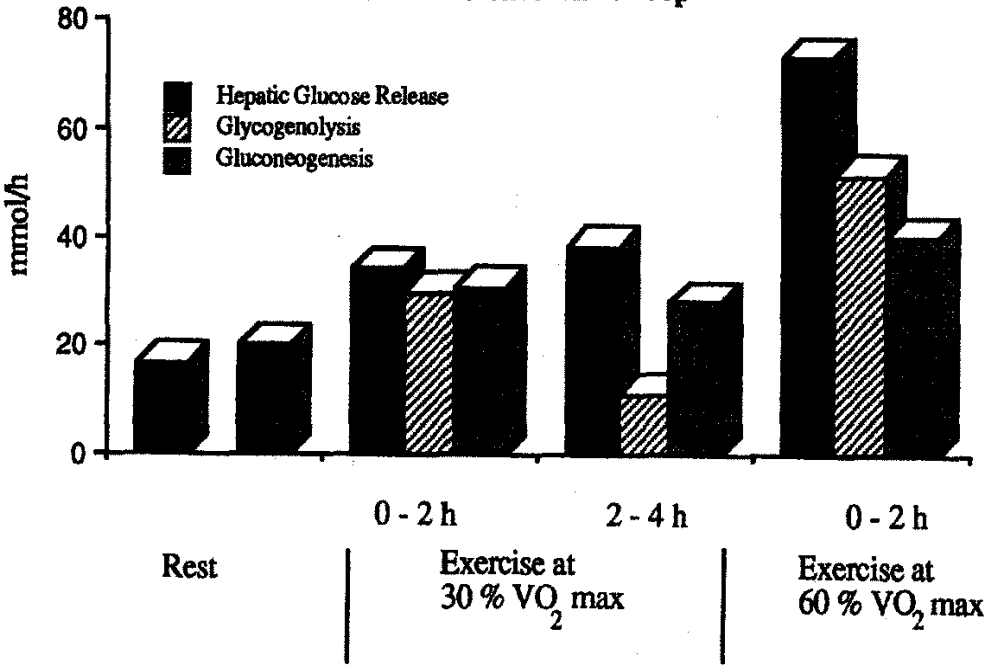
Table 2. Glucose metabolism in sheep during exercise

Level of Exercise	Glucose [a] Concn. (mM)	Hepatic Release (mmol h^{-1})	Muscle [b] Uptake (mmol h^{-1})	Contr. to [c] Muscle O_2 (%)	Relative Energy Use [g] (kg^{-1})
Rest (7) [d]	3.0±0.1	17±2	1.1±0.2	54±9	1.0
30%VO_2max [e] (10)	2.8±0.2	35±5	2.4±1.0	24±6	2.4
60%VO_2max [f] (7)	3.3±0.3	73±13	4.8±0.8	31±5	4.2

Results are presented as mean±SEM of the exercise period, mean sheep weight was 40kg
a Arterial concentration at the end of exercise, no significant differences between values
b Hindlimb muscle preparation
c Assuming complete oxidation, allowing for output of lactate and pyruvate.
d Numbers of animals
e Below the anaerobic threshold. VO_2max figures are estimates based on maximal O_2 consumption of 7x over rest (Lotgering et al.1983)
f Above the anaerobic threshold, blood lactate concentration was 4.5mM
g Determined from the entry rate of carbon dioxide

The reason for the different responses are most probably related to different rates of gluconeogenesis. Glucose released by the liver is derived from either glycogenolysis or gluconeogenesis. Figure 1 shows the rates of these two pathways for sheep exercised according to the protocol described for Table 2.

Fig 1. **Potential Sources of Hepatic Glucose Release in Sheep**



There was significant glycogenolysis at both levels of exercise which resulted in substantial depletion of hepatic glycogen (70-80% depletion, Pethick and Harman 1989). However the potential for gluconeogenesis was large (determined by assuming complete conversion of gluconeogenic substrate utilised by the liver to glucose), such that at the lower level of exercise nearly all the hepatic glucose release could be accounted for, while at 60% VO_2max the potential contribution dropped to 60%. Comparable calculations derived from the data of Ahlborg (cited above) suggest that the average maximum potential for gluconeogenesis was at best 30% of the glucose released by the liver in the human athlete. This represents a rate of gluconeogenesis (expressed as mmol h^{-1} kg BW^{-1}) some 50% below that found for sheep. Consequently glycogen depletion would occur more quickly in the human athlete.

(c) Glycogen Metabolism

Glycogen in skeletal muscle is also a fuel for exercise. The rate of use is again dependent on work rate. In sheep, exercise below the anaerobic threshold resulted in no significant depletion of glycogen in muscle. However above the anaerobic threshold glycogen depletion became significant (Pethick and Harman 1989). In more comprehensive studies utilising human athletes, glycogenolysis in muscle rises rapidly in a curvilinear manner at work rates above the anaerobic threshold (Saltin and Karlsson 1971).

The rate and control of glycogen repletion in ruminants is not fully understood. This is surprising when one considers the importance of glycogen for meat quality, thermoregulation (Martineau and Jacobs 1988) and coping with stress. Recent work in my laboratory has measured glycogen repletion in sheep adapted to exercise (Table 3). The rate of repletion in liver tissue is slower (some 3-6 times) than in rats (Shulman et al.1991; Holness et al.1988) or human subjects (Nilsson and Hultman 1974). However this is expected since the nonruminant work involved fasting followed by refeeding a high carbohydrate diet or administration of glucose. In nonruminants, particularly in the refeeding model, about 40% of the glycogen is derived from glucose via the glucokinase reaction (the direct pathway) and the remainder via gluconeogenesis (indirect pathway) using 3 carbon metabolites derived from the refed carbohydrate (i.e. the glucose paradox, Katz and McGarry 1984). In contrast ruminants on most diets could only synthesise glycogen via the indirect gluconeogenic route with propionate as the main substrate. Thus while relatively slow, the sheep is quite capable of modest rates of glycogen synthesis on roughage diets fed to maintenance. The extent to which nutrition can modify this rate is unknown.

Comparative rates of glycogen repletion in muscle are more complex to understand. The rate depends on muscle type, being typically most rapid in type IIA or fast twitch, oxidative muscle and least rapid in type IIB or fast twitch, glycolytic type muscle (Conlee et al. 1978). Recent experiments (Table 3) show that sheep follow the same pattern since the semimembranosis and semitendinosis muscles are regarded as predominantly type IIB and IIA respectively (Monin 1980; Briand et al.1981). Other studies in sheep (Monin 1980) and cattle (McVeigh et al. 1982) have shown much lower (some six fold) rates of repletion. These differences are probably explained by the effects of stress (cited as a complication in the work of Monin 1980), training (the cattle in the McVeigh et al.1982 study were tethered), time period of study and muscle type. Muscle from rats trained for exercise has around twice the ability to replete glycogen when compared to sedentary controls (Tan et al. 1984). The rates of repletion in sheep for the semimembranosis muscle (table 3) are close to those found in human athletes for the vastus lateralis muscle (values range from 4-7mmol kg^{-1} h^{-1}, Maehluim et al. 1977; Ivy et al. 1988) and higher than for the gluteal muscle of the horse (1-1.5mmol kg^{-1} h^{-1}, Hodgson 1981). Clearly sheep adapted to exercise have a considerable capacity to replete glycogen in some muscle groups, especially when the nutritional differences are accounted for.

Table 3. Glycogen repletion in sheep post exercise

Tissue	Rate (mmol glucose units kg^{-1} h^{-1})
Liver	8.8±1.1 (9)[a]
Semimembranosis muscle	3.7±0.8 (8)
Semitendinosis muscle	1.1±0.7 (9)

a - number of sheep.

Exercise was walking at 4.5km/h on 9° incline for 2h. Biopsy samples were taken at the end of exercise and then the sheep were euthanased at either 3 or 6h post exercise and further samples taken. Sheep were fed a diet of lucerne cubes feed to maintenance semicontinuously.

IV. METABOLISM OF FATTY ACIDS

Exercise in sheep prompts a dramatic shift to fat metabolism. Table 4 shows various parameters of fat metabolism in sheep exercised according to the protocol described for Table 2. The increase in fat turnover, if fully oxidised, could account for all of the energy metabolism. However much of the NEFA are not oxidised in the short term since only around 30% can be recovered as CO_2 during the exercise period, indicating substantial re-esterification of fatty acids (Pethick et al. 1987). At the lower work rate the concentration of fatty acids increased slowly throughout the 4 hours of exercise while at the higher level of exercise peak concentrations were reached within 30min. The contribution of circulating NEFA to the energy metabolism of skeletal muscle ranged from 40-70%, with the higher values being found toward the end of exercise when NEFA concentration was highest.

Table 4. Metabolism of Fatty acids in sheep during exercise

Level of Exercise	Conc. Blood NEFA (mM)	Entry Rate NEFA (mmol h^{-1} kg^{-1})	Contribution [a] to Energy Expenditure (%)	Conc. Ketone Bodies (mM)	Conc. Blood Acetate (mM)
Rest (6)[b]	0.1±0.01	0.1±0.02	14	0.4±0.01	1.2±0.2
30%VO_2max (6)	0.8±0.1 [c]	1.7±0.1 [c]	117	0.5±0.03 [c]	1.5±0.2
60%VO_2max (6)	1.2±0.2 [c,d]	3.0±0.1 [c,d]	122	0.5±0.03 [c]	2.3±0.3 [c]

Results are mean±SEM and represent the mean of the exercise period. Values for concentration represent arterial blood.

a Contribution to whole body CO_2 entry rate assuming complete oxidation.

b number of animals

c significantly different to rest ($P<0.05$)

d significantly different to 30% VO_2max ($P<0.05$)

Partial oxidation of fatty acids to ketone bodies does not represent an important pathway for fat metabolism. This is despite a substantial increase in the uptake of NEFA by the liver and suggests that special mechanisms exist to inhibit ketogenesis. A similar situation is found during exercise in the human athlete (Wahren et al.1984). Furthermore the contribution made by ketones to the energy metabolism of exercising muscle is very low at 5% for sheep and less than 1% for the human. The mechanism for reduced ketogenic capacity during exercise may be increased sympathetic nerve activity which is known to inhibit ketogenesis (Beuers et al. 1986). In fed ruminants methylmalonylCoA could also contribute to the inhibition of ketogenesis during exercise (Brindle et al. 1985).

In contrast, sustained exercise at above the anaerobic threshold prompts an increase in the concentration of acetate in the blood of sheep. The net result is extra fuel available to skeletal muscle such that acetate can account for around 10% of the energy expenditure of skeletal muscle during sustained work. The differential control of NEFA conversion to either ketone bodies or acetate is yet to be understood but it may involve peroxisomal oxidation of fatty acids. The role of acetate during exercise in nonruminant animals has not been studied.

V. CONCLUSION

(a) Athletic Performance

This paper points to further research into the extent and control of gluconeogenesis in athletic animals and man. It might be that gluconeogenesis in a well trained distance runner is substrate limited, if this were the case, consumption of a gluconeogenic precursors might slow carbohydrate depletion during a race. Alternatively strategies might be devised to induce gluconeogenesis without compromising the need for carbohydrate intake. More work is needed to see if the partial oxidation of NEFA to short chain acids can be increased. This would increase the power of fat oxidation by converting fat into water soluble, highly oxidisable fuel. A possible axis for research in this area might be to optimise peroxisomal oxidation of NEFA.

(b) Role of exercise in sheep nutrition

This subject still remains incompletely understood. Clearly exercise will increase the requirement for metabolisable energy, but more importantly the animal will require a greater release of gluconeogenic end products of fermentation to act as sources of increased glucose production. This would be of little consequence on good quality feed but during the long Australian summer it could lead to excessive weight loss as animals exercise to search for feed. The interaction of feed quality, exercise and foraging ability has yet to be fully understood. The extra requirement for fat during exercise might also play an important role in body composition of sheep kept under different management conditions. Finally there is a need to further study the control and extent of glycogen metabolism under different nutritional regimes and examine the role played by exercise in upregulating this process in ruminants.

REFERENCES

AHLBORG, G and FELIG, P. (1982). J. Clin. Invest. 69: 45.

AHLBORG, G., FELIG, P., HAGENFELDT, L., HENDLER, R. and WAHREN, J. (1974). J. Clin. Invest. 53: 1080.

BEUERS, U., BECKH, K., and JUGERMANN, K. (1986). Eur. J. Biochem. 150: 19.

BRIAND, M., TALMANT, A., BRIAND, Y., MONIN, G. and DURAND, R. (1981). Eur. J. Appl. Physiol. 46: 347.
BRINDLE, N.P.J., ZAMMIT, V.A., and POGSON, C.I. (1985). Biochem. J. 232: 177.
HARMAN, N.G. (1991). PhD Thesis, Murdoch University, Western Australia.
HARMAN, N.G. and PETHICK, D.W. (1987). Proc. Nutr. Soc. Aust. 12: 35.
HODGSON, D.R. (1981). PhD Thesis, University of Sydney, New South Wales, Australia.
IVY, J.L., LEE, M.C., BROZINICK, J.T. and REED, M.J. (1988). J. Appl. Physiol. 65: 2018.
KATZ, K. and McGARRY, J.D. (1984). J. Clin. Invest. 74: 1901.
KJAER, M., SECHER, N.H., BACH, F.W. and GALBO, H. (1987). Am. J. Physiol. 253: R687.
LEURY, B.J., BIRD, A.R., CHANDLER, K.D. and BELL, A.W. (1990). Br. J. Nutr. 64: 449.
LOTGERING, F.K., GILBERT, R.D. and LONGO, L.D. (1984). Am. J. Obstet. Gynecol. 5: 560.
LOTGERING, F.K., GILBERT, R.D. and LONGO, L.D. (1985). Physiol. Rev. 65: 1.
MAEHLUM, S., HOSTMARK, A.T. and HERMANSEN, L. (1977). Scan. J. Clin. Lab. Invest. 37: 309.
MARTINEAU, L. and JACOBS, I. (1988). J. Appl. Physiol. 65: 2046.
MAZZEO, R.S. and MARSHALL, P. (1989). J. Appl. Physiol. 67: 1319.
McVEIGH, J.M., TARRANT, P.V. and HARRINGTON, M.G. (1982). J. Anim. Sci. 54: 790.
MESZAROS, K., LANG, C.H., HARGROVE, D.M. and SPITZER, J.J. (1989). J. Appl. Physiol. 67: 1770.
MONIN, G. (1980). In "The Problem of Dark Cutting Beef". Current Topics in Veterinary Medicine and Animal Science, Vol 10, p.63, eds Hood, D.E. and Tarrant, P.V. (Martinus Nijhoff Publishers: Netherlands).
NILSSON, L.H. and HULTMAN, E. (1974). Scand. J. Lab. Invest. 33: 5.
PETHICK, D.W. and HARMAN, N.G. (1989). Proc. 7th International Conference on Production Disease in Farm Animals, p61, (Cornell Uni.: U.S.A.).
PETHICK, D.W., HARMAN, N.G. and CHONG, J.K. (1987). Aust. J. Biol. Sci. 40: 331.
PETHICK, D.W., MILLER, C.M. and HARMAN, N.G. (1991). Aust. J. Agric. Res. 42: 599.
SAHLIN, K. (1986). In "Biochemistry of Exercise VI", International Series on Sport Sciences, Vol 16, p.323, ed. Saltin, B. (Human Kinetics Publ.: Illinois).
SALTIN, B. and KARLSSON, J. (1971). Adv. in Medicine and Biology 11: 289.
SHULMAN, G.I., DeFONZO, R.A. and ROSSETTI, L. (1991). Am. J. Physiol. 260: E731.
SONNE, B., MIKINES, K.J. and GALBO, H. (1987). Am. J. Physiol. 252: R587.
TAN, M.H., BONEN, A., WATSON-WRIGHT, W., HOOD, D., SOPPER, M., CURRIE, D., BELCASTRO, A.N. and PIERCE, G. (1984). J. Appl. Physiol. 57: 1404.
VISSING, J., SONNE, B. and GALBO, H. (1988). J. Appl. Physiol. 65: 2552.
WAHREN, J., SATO, Y., OSTMAN, J., HAGENFELDT, L. and FELIG, P. (1984). J. Clin. Invest. 73: 1367.