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# Glucose and Acetate Metabolism in Sheep at Rest and During Exercise

*G. J. Judson*,[A] *O. H. Filsell*[B] *and I. G. Jarrett*[B]

[A] Institute of Medical and Veterinary Science, Frome Road, Adelaide, S.A. 5000.
[B] Division of Human Nutrition, CSIRO, Kintore Avenue, Adelaide, S.A. 5000.

*Abstract*

Total entry rate of blood glucose and the rate of irreversible loss of blood acetate and its oxidation have been examined in sheep at rest and while walking on a horizontal treadmill at 5 km/h for 2 h. Sheep were given their daily ration of 1000 g chaff in 24 equal portions at hourly intervals and received multiple intravenous injections of [2-$^3$H]glucose and intravenous infusions of [1-$^{14}$C]acetate and $NaH^{14}CO_3$.

At rest the total entry rate of blood glucose was 0·44 ± 0·03 mmol/min (values given as mean ± s.e.m. for four sheep), the glucose pool was 23 ± 1 mmol and the rate of irreversible loss of blood acetate was 2·3 ± 0·1 mmol/min. During exercise, the total entry rate of blood glucose was 0·84 ± 0·04 mmol/min, the glucose pool was 27 ± 2 mmol and the rate of irreversible loss of blood acetate was 2·6 ± 0·1 mmol/min.

Gluconeogenesis apparently increased markedly in response to exercise as indicated by the incorporation of $^{14}$C from blood bicarbonate into blood glucose. Despite the substantial increase in the rate of irreversible loss of blood bicarbonate (from 11·6 ± 1 to 20·2 ± 2 mmol C/min), and hence energy expenditure with exercise, only a slight change was recorded in the proportion of the irreversible loss rate of acetate that was oxidized.

## Introduction

Adult ruminants derive the greater part of their energy requirements from short-chain fatty acids which are largely produced in the rumen by microbial fermentation of ingested carbohydrate. In contrast to monogastric animals little dietary glucose is absorbed as such, the glucose requirements being provided by gluconeogenesis largely from propionate and from glucogenic amino acids.

Of the short-chain fatty acids only acetate, which is probably not utilized to any extent in the liver, is present in significant amounts in peripheral blood for utilization by extra-hepatic tissue (Bergman and Wolff 1971). Estimates based on isotope-dilution techniques indicate that blood acetate contributes about 20% to the total energy expenditure in fed sheep whereas glucose contributes only about 7% (Annison *et al.* 1967).

The relative importance of glucose and acetate as sources of energy for the hind limb of sheep at rest and during exercise has been examined by Jarrett *et al.* (1976). They report that the utilization of glucose was greatly increased by exercise, the glucose supplying about 27% of the fuel of respiration for the exercising limb whereas acetate supplied only about 2%. In the resting hind limb little utilization of glucose was recorded although acetate oxidation accounted for about 20% of the oxygen uptake. These observations were made by measurements of blood flow and

arterio–venous differences across the hind limb of the sheep and thus represent changes taking place predominantly in hind limb muscles.

In view of these observations the role of glucose and acetate metabolism in the whole animal at rest and during exercise was examined by isotope-dilution techniques.

## Materials and Methods

*Animals and Diet*

The eight Merino wethers used in this study were about 2 years old and weighed between 35 and 40 kg. They were kept in single metabolism crates and were given 500 g lucerne hay chaff and 500 g wheaten hay chaff each day for at least 4 weeks before these studies commenced. The daily ration was given in 24 equal portions at hourly intervals to approximate steady-state conditions, particularly with respect to the supply of exogenous acetate and gluconeogenic substrates.

All animals were accustomed to frequent handling and to walking on a horizontal moving belt exercise machine at a speed of 5 km/h. Experiments with these animals consisted of a 3- or 4-h rest period followed by 2 h of exercise. The feeding regime was maintained through this experimental period.

*Experimental Procedure*

The day before an experiment, polyvinyl catheters were inserted into both jugular veins, one to be used for infusing [1-$^{14}$C]acetate and $NaH^{14}CO_3$ and the other for obtaining blood samples and for injecting [2-$^{3}$H]glucose. Four sheep received a [1-$^{14}$C]acetate infusion and 3 days later they received an $NaH^{14}CO_3$ infusion. Two of these sheep were given multiple injections of [2-$^{3}$H]glucose during the [1-$^{14}$C]acetate infusion and the other two were given the multiple injections of [2-$^{3}$H]glucose during the $NaH^{14}CO_3$ infusion.

[1-$^{14}$C]Acetate (0·38–0·41 $\mu$Ci/min) was infused for 5 h following a priming dose of 50 $\mu$Ci [1-$^{14}$C]acetate, and $NaH^{14}CO_3$ (0·33–0·65 $\mu$Ci/min) was infused for 6 h following a priming dose of 50 $\mu$Ci $NaH^{14}CO_3$. The animals were exercised during the last 2 h of the isotope infusions and successive multiple injections of [2-$^{3}$H]glucose were given over the last 4 h of the isotope infusions. The first injection of [2-$^{3}$H]glucose (14·6–18·9 $\mu$Ci) was given approximately 2 h before the start of exercise, the second injection (51·6–70·2 $\mu$Ci) during the first hour of exercise and the third injection (190–241 $\mu$Ci) during the second hour of exercise. A further four sheep also received the [1-$^{14}$C]acetate but the isotope was infused for only 4 h following the priming dose of [1-$^{14}$C]acetate and the sheep were exercised during the last 2 h of the infusion.

Samples of blood were taken at 15–20-min intervals during a 1–2-h period before exercise and also during exercise. Blood samples taken before exercise were used to obtain resting values for the concentration and specific radioactivity (SR) of circulating substrates.

*Chemical Methods*

(i) *Assay of radioactive glucose*

Blood samples for glucose and acetate analysis were immediately added to tared tubes containing iced water, the tubes were reweighed to record the weight of blood added and the diluted blood was deproteinized by the method of Somogyi (1945). About 5 ml blood were taken for glucose analyses and about 6 ml blood for acetate analyses.

Glucose concentration and radioactivity in the supernatant were determined by the method of Huggett and Nixon (1957) and Jones (1965) respectively. Measurement of the radioactivity of the penta-acetate derivative of glucose was carried out using a Tri-Carb liquid scintillation spectrometer (model 3375, Packard Instrument Co. Ltd) and the external standard was calibrated to correct for quenching. Efficiencies of counting of 40% were usually obtained for $^{3}$H and $^{14}$C, with about 35% of the $^{14}$C appearing in the $^{3}$H channel.

(ii) *Assay of radioactive acetate*

Acetate concentration in the supernatant from deproteinized blood was determined by gas–liquid chromatography. An aliquot of the supernatant was made alkaline with NaOH, evaporated to dryness under a stream of warm air, the residue reconstituted in 10% (w/v) $H_3PO_4$, and 5 $\mu$l of the solution applied to the column. The column packing was Chromosorb 101, mesh

80–100 (Johns Manville Celite Division, Denver, Co., U.S.A.), operating temperature 175°C, flame ionization detector temperature 220°C, injection port temperature 220°C and carrier gas flow rate 32 ml $N_2$/min. The recovery of a standard mixture of short-chain fatty acids using the above procedure was quantitative. Sodium isobutyrate added to the protein-free supernatants was used as an internal standard.

The radioactivity of acetate was determined using the remainder of the protein-free supernatant. This known volume of supernatant was freeze-dried after the addition of carrier sodium acetate (0·04 mmol) and [2-$^3$H]acetic acid (16·5 nCi). The dried residue was acidified and the acetate isolated using a silica-gel column (Ramsey 1963) and a hexane–butanol mixture (Oppermann *et al.* 1957) as the eluting solvent. The acetate was extracted from the solvent with 3 ml 0·3 M KOH and 2 ml of the alkaline extract was prepared for assay of radioactivity by mixing with 20 ml Triton X-100 scintillation mixture (Patterson and Greene 1965). The radioactivity was measured in the Tri-Carb. Efficiencies of counting of 20 and 35% were usually obtained for $^3$H and $^{14}$C respectively, with about 35% of the $^{14}$C appearing in the $^3$H channel. The recovery of $^3$H in the sample was used to correct for the recovery of $^{14}$C-labelled acetate from the protein-free supernatant.

(iii) *Assay of radioactive bicarbonate*

Blood bicarbonate was isolated and assayed for radioactivity as barium carbonate by the method of Leng and Leonard (1965) except that the $H_2SO_4$ injected into blood contained $CuSO_4$ (10 g/l) to prevent bacterial action.

(iv) *Radioactive compounds*

Sodium [1-$^{14}$C]acetate, [2-$^3$H]acetic acid, $NaH^{14}CO_3$ and [2-$^3$H]D-glucose were obtained from the Radiochemical Centre, Amersham, England.

The isotopes to be infused were made to volume with sterile Ringer's solution containing about 0·3 mmol/l of the non-labelled substrate as a carrier. These solutions were either infused at 0·2 ml/min using a pump (model Unita I, B. Braun-Melsungen) driving the plunger of a 50-ml syringe or known volumes given as a single injection of about 5 ml. Samples of these solutions were prepared for assay of radioactivity as described for blood substrates. Aliquots of the $NaH^{14}CO_3$ solution, however, were diluted with 2·0% (w/v) $NaHCO_3$ in $CO_2$-free water before assaying for radioactivity. Aliquots of this solution were also recounted with $^{14}$C standard in Triton X-100 scintillation mixture in order to check recovery of $^{14}$C when assayed as $Ba^{14}CO_3$.

*Calculations*

The total entry rate and pool size of glucose were calculated from the initial part of the decay curve of [2-$^3$H]glucose in blood glucose as described by Judson and Leng (1972). The zero-time intercept of the decay curve for the second and third injections of [2-$^3$H]glucose was corrected for residual tritium activity. This residual tritium in the glucose pool was found to be less than 5% of the tritium injected. The turnover time of the glucose pool was obtained by dividing the pool size by the total entry rate of glucose.

The rate of irreversible loss of metabolite was calculated by comparing the 'plateau' SR of the metabolite with the rate of infusion of the labelled metabolite. This technique, which was used in the present study to measure the effect of exercise on the rates of irreversible loss of acetate and bicarbonate, has been used previously to measure short-term changes in the rate of irreversible loss of blood metabolites (Judson and Leng 1973).

From the data obtained with the four sheep given both the [1-$^{14}$C]acetate and $NaH^{14}CO_3$ infusions, the proportion of blood acetate produced which was oxidized was calculated. To obtain this value for each sheep the plateau SR value for blood bicarbonate-carbon (nCi/mmol C) observed during infusion of [1-$^{14}$C]acetate was divided by the infusion rate of [1-$^{14}$C]acetate (nCi/min) and multiplied by the irreversible loss of blood bicarbonate-carbon (mmol C/min).

## Results

### *Metabolism of Glucose*

A typical experiment on the metabolism of glucose during exercise is shown in Fig. 1. The linear decline in the log SR of blood glucose with time between about 20

and 90 min after injection of [2-$^3$H]glucose was observed in all experiments. The SR of blood bicarbonate-carbon during infusions of $NaH^{14}CO_3$ was constant during rest, but exercise resulted in a rapid decrease in the bicarbonate SR for 10–30 min before it fell to a new plateau (Fig. 1). Similar trends in the SR of glucose-carbon were also observed during these $NaH^{14}CO_3$ infusions except that during exercise a constant glucose SR was not obtained until during the final 30 min of exercise. There was little fluctuation in blood glucose concentration during rest or exercise (see Fig. 1).

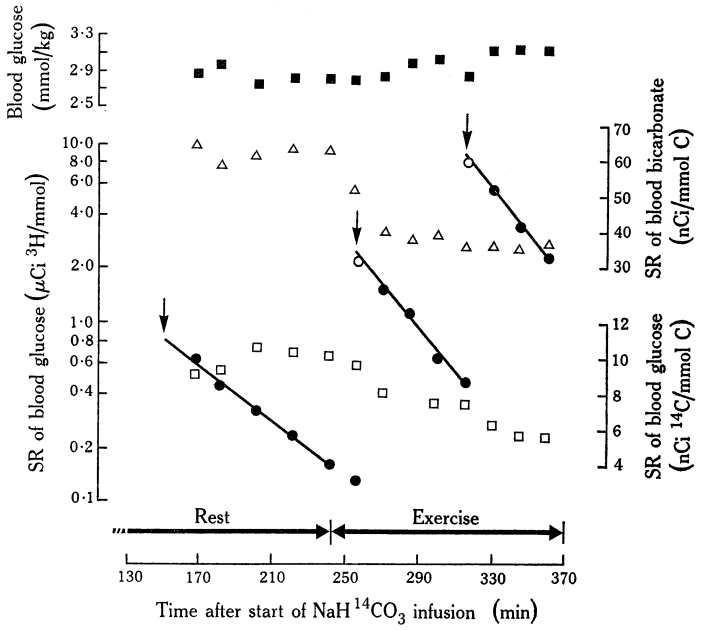


**Fig. 1.** Effect of exercise on the SR of blood glucose and blood bicarbonate and the concentration of blood glucose in sheep 2602 given multiple intravenous injections of [2-$^3$H]glucose during an intravenous infusion of $NaH^{14}CO_3$ (653 nCi/min). □ SR of glucose (nCi $^{14}$C/mmol C). ● SR of glucose (μCi $^3$H/mmol). ○ Adjusted zero-time SR of glucose (μCi $^3$H/mmol). △ SR of blood bicarbonate. ■ Concentration of glucose. The first (18·9 μCi), second (51·6 μCi) and third (200 μCi) injections of [2-$^3$H]glucose were given at 150, 257 and 317 min respectively after the start of the infusion of $NaH^{14}CO_3$, and are shown by arrows.

Table 1 summarizes the results obtained. The small increase in the mean blood glucose concentration and glucose pool size usually observed with exercise contrasts with the marked increase in the total entry rate of blood glucose and the concomitant decrease in the turnover time of the glucose pool (Table 1). The source of this extra glucose was examined in the two sheep given infusions of $NaH^{14}CO_3$ together with injections of [2-$^3$H]glucose by using the incorporation of $^{14}$C from blood bicarbonate into glucose as an index of gluconeogenesis (see Judson and Leng 1973). During rest the proportion of glucose-carbon derived from bicarbonate-carbon was about 16% but with exercise it decreased from 16·6 to 15·5% in one sheep and from

16·3 to 8·8% in the other sheep. Despite these decreases, however, the calculated rate of incorporation of glucose-carbon from blood bicarbonate increases markedly with exercise in both sheep (Table 1).

**Table 1. Effect of exercise on glucose metabolism**

Values expressed are means ± s.e.m. for four sheep

| Blood glucose (mM) | Glucose pool (mmol) | Turnover time (min) | Total entry rate of glucose (mmol/min) | Irreversible loss of bicarbonate (mmol C/min) | Glucose-C derived from bicarbonate[C] (%) | (mmol/min) |
|---|---|---|---|---|---|---|
| | | | Rest | | | |
| 2·8±0·1[A] | 23±1 | 52±3[AB] | 0·44±0·03[AB] | 11·6±1·0[B] | 16 | 0·38 |
| | | | Exercise for 2 h[D] | | | |
| 3·1±0·1[A] | 27±2 | 32±1[A] | 0·84±0·04[B] | 20·2±2·0[B] | 12 | 0·57 |
| (3·0±0·1) | (27±2) | (33±1[B]) | (0·83±0·08[A]) | | | |

[A] Values significantly different from each other at $P < 0·05$.
[B] Values significantly different from each other at $P < 0·01$.
[C] Mean values for two sheep.
[D] Parameters of glucose metabolism during the first hour of exercise are given in parentheses.

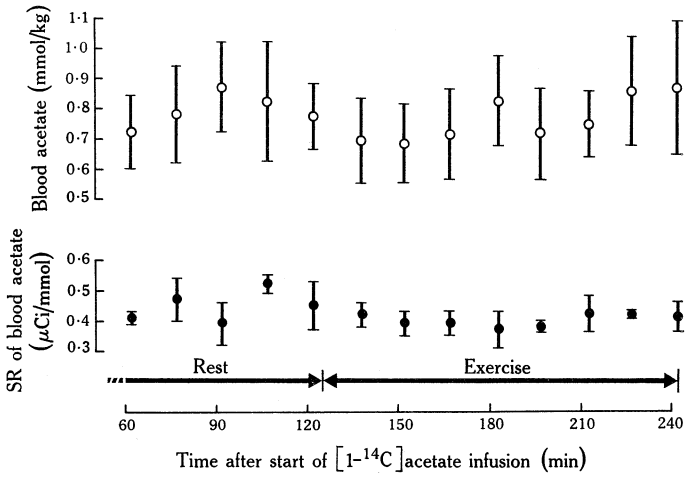


**Fig. 2.** SR and concentration of blood acetate (mean ± s.e.m.) before and during exercise in four sheep. The [1-$^{14}$C]acetate was infused intravenously and the SR of blood acetate was adjusted to an infusion rate of 1 μCi/min.

*Metabolism of Acetate*

The concentration and SR of acetate in jugular blood of sheep given infusions of [1-$^{14}$C]acetate were approximately constant during rest and exercise. Changes in these parameters with exercise were similar for the four sheep studied and Fig. 2 gives the mean values and the ranges about the means for the concentration and SR of acetate. In two of these sheep the blood acetate concentration was about 0·5 mmol/l whereas the other two had acetate concentrations of about 1·0 mmol/l. These differences, which were not reflected in the SR values (when adjusted for similar

infusion rates of isotope), accounted for the large standard errors shown for acetate concentration in Fig. 2.

The rate of irreversible loss of blood acetate increased slightly in all sheep with exercise but this change was not significant when examined by the paired *t* test (Table 2). The blood acetate concentration was also not significantly affected by exercise ($P > 0{\cdot}05$) although in three of the four sheep the mean concentration during exercise was less than the mean resting value. A slight increase was recorded in the rate of oxidation of blood acetate with exercise but its contribution to total energy expenditure apparently decreased, as indicated by the proportion of blood bicarbonate derived from acetate-carbon (Table 2).

**Table 2. Effect of exercise on acetate metabolism**

Values expressed are means ± s.e.m. for four sheep. For columns 1–3 mean values were not significantly different ($P > 0{\cdot}05$) from each other

| Blood acetate (mM) | Irreversible loss of acetate (mmol/min) | Acetate produced and oxidized[A] (%) | Rate of oxidation of acetate[B] (mmol C/min) | Blood bicarbonate-C derived from acetate[B] (%) |
|---|---|---|---|---|
| | | Rest | | |
| 0·79±0·14 | 2·3±0·1 | 58±3 | 2·7 | 23 |
| | | Exercise for 2 h | | |
| 0·76±0·14 | 2·6±0·1 | 62±4 | 3·2 | 16 |

[A] This represents the proportion of the irreversible loss rate of acetate that was oxidized.
[B] Calculated using the mean values for acetate produced and oxidized (column 3 this table) and rate of irreversible loss of blood bicarbonate (Table 1).

## Discussion

In the present study of glucose and acetate metabolism by using isotope-dilution techniques the total entry rate of blood glucose, the irreversible losses of blood acetate and bicarbonate, the rate of incorporation of blood bicarbonate-carbon into blood glucose and the rate of oxidation of acetate have been determined in the whole animal at rest and during 2-h periods of moderate physical activity. These observations extend those reported by Jarrett *et al.* (1976) and indicate that while the sheep was walking horizontally at 5 km/h there was approximately a twofold increase in the total entry rate of glucose which persisted throughout the period of exercise. Similar responses in glucose production to moderate exercise have also been recorded by isotopic techniques in other species including man (Young *et al.* 1967) and dog (Issekutz *et al.* 1967; Paul and Issekutz 1967; Vranic and Wrenshall 1969). The total entry rate of glucose as measured in the present work with [2-$^3$H]glucose largely represents the total hepatic output of glucose (see Judson and Leng 1972). Only small quantities of blood glucose are produced by the kidney (McIntosh *et al.* 1973) or absorbed from the alimentary tract of sheep given roughage (MacRae and Armstrong 1966).

The relative importance of the gluconeogenic and glycogenolytic pathways in contributing to the hepatic output of glucose was examined in this study by using the incorporation of bicarbonate-$^{14}$C into glucose as an index of gluconeogenesis (Judson and Leng 1973). The rate of gluconeogenesis apparently increased in res-

ponse to exercise as indicated by the marked increase in the rate of incorporation of bicarbonate-$^{14}C$ into blood glucose. Whether this increase was due to a more efficient utilization of gluconeogenic substrates or to a greater availability of these substrates from peripheral tissues is not known. However, in one sheep the increased rate of gluconeogenesis did not account for all the glucose, some presumably being derived from glycogen, as indicated by a drop in the proportion of glucose-carbon derived from bicarbonate (Table 1). It seems reasonable to assume that glycogen could supply much of the extra glucose produced during exercise, as liver concentrations of glycogen in fed sheep are from 170 to 280 mmol/kg (Ford 1962). In man during moderate exercise, the initial production of blood glucose is apparently derived largely by mobilization of liver glycogen but gluconeogenesis appears to provide an increasing fraction of this glucose with continued exercise (Young *et al.* 1967; Wahren *et al.* 1971; Ahlborg *et al.* 1974).

Estimates of glucose oxidation in man and the dog show that most of the glucose which is produced during moderate exercise is oxidized and provides about 10–20% of the total energy requirements (Issekutz *et al.* 1967; Paul and Issekutz 1967; Young *et al.* 1967). As indicated earlier (see Jarrett *et al.* 1976) arterio–venous differences show that large amounts of glucose are taken up by the exercising hind limb of sheep and as there is no significant release of lactate it is probable that most of the glucose is oxidized. Thus from estimates based on the blood glucose produced during exercise (see Table 1) it appears that glucose could contribute about 20% of the $CO_2$ produced—indicating that a comparable situation exists between sheep and man or dog.

Although acetate is available in sufficiently large amounts from the rumen to account for a substantial proportion of the energy requirements of sheep it is a very transient metabolite with a half-time of 3–4 min for blood acetate-$^{14}C$ (Annison and Lindsay 1961). Data from Jarrett *et al.* (1976) show that, at rest, direct oxidation of acetate can account for 20% of the oxygen uptake of the hind limb in fed sheep, but that during exercise it is a minor nutrient accounting for 2% or less of the oxygen uptake of the hind limb. This low uptake of acetate by the hind limb during exercise is related to small arterio–venous differences which in turn are dependent at least in part on the lower arterial level of acetate during exercise.

The irreversible loss of acetate reported here for normal resting sheep is similar to values reported by Annison *et al.* (1967) and Bergman and Wolff (1971). In response to exercise the irreversible loss of blood acetate and its rate of oxidation were unchanged, suggesting that a significant amount of the acetate was utilized by tissues other than by exercising muscles.

The present results suggest that acetate is a minor source of energy for exercising muscles and although glucose production increases it accounts for only 20% of the energy required. Thus during moderate exercise sheep rely on other substrates, probably free fatty acids, for a major source of energy as shown for the hind limb (Jarrett *et al.* 1976).

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