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In vivo measurement of lipogenesis in ruminants using [1-^{14}C]acetate

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A method for the measurement of the rate of lipogenesis in ruminants using a continuous intravenous infusion of [1-^{14}C]acetate and measuring the rate of [1-^{14}C]acetate incorporation into adipose tissue lipid was evaluated. Subcutaneous adipose tissue samples obtained by biopsy over the course of a 6 h continuous intravenous infusion of [1-^{14}C]acetate into a wether and a steer maintained in a 'metabolic steady state' demonstrated that the incorporation of [1-^{14}C]acetate into subcutaneous adipose tissue lipid was linear for the duration of the infusion period. Subsequent measures of rates of [1-^{14}C]acetate incorporation into adipose tissue lipid were made on adipose tissue samples taken at a single time point during the infusion period. The technique was used to measure rates of lipogenesis in the subcutaneous adipose tissue of fourteen Hereford × Friesian steers that had been fed a pelleted diet of dried grass at a range of metabolizable energy (ME) intakes from 1·1 × ME requirement for maintenance to *ad libitum* for 11 weeks. Rates of lipogenesis increased linearly ($P < 0{\cdot}001$) with increasing ME intake. It was concluded that the method is an effective technique for measuring rates of lipogenesis in specific adipose tissue depots *in vivo* in ruminants.

Ruminant: Lipogenesis: Acetate: Metabolizable energy intake

Ruminant diets are typically low in fat (20–50 g/kg), as high-fat diets impair rumen function (Bauman, 1976). As a result, the gastrointestinal absorption of preformed lipids is usually considerably lower in normally fed ruminants than in non-ruminants. Long-chain fatty acids synthesized *de novo* are therefore an important source for triacylglycerol synthesis in ruminant adipose tissue, making the rate of fatty acid synthesis (lipogenesis) an important kinetic variable for the study of lipid metabolism in ruminant animals.

Most reported rates of lipogenesis in ruminant animals have come from *in vitro* measurements using adipose tissue incubations. This technique is useful for examining direct hormonal effects on adipose tissue metabolism. However, the reliability of the quantitative data has been questioned (Mersmann, 1986; Dunshea *et al.* 1992), as the adipose tissue in *in vitro* incubations tends to be in a net degradative state, resulting in overestimated rates of lipolysis and underestimated rates of lipogenesis.

Measurements of lipogenesis *in vivo* have been made in sheep using both a single injection (Ingle *et al.* 1972*b*) and continuous infusion (Broad & Ulyatt, 1980) of radiolabelled acetate. The quantitative incorporation of label into lipid using these methods was used to demonstrate that adipose tissue was the most important site of lipogenesis in ruminants. However, these methods do not allow dynamic measurements of the rates of lipogenesis to be made.

Dynamic measurements of triacylglycerol–glycerol synthesis have been made in sheep using continuous infusions of [U-^{14}C]glucose (Davey, 1986). This method was extended to measure rates of *de novo* fatty acid synthesis (lipogenesis) in pigs (Dunshea *et al.* 1992). Here, an *in vivo* method for measuring the rate of lipogenesis in ruminants using a continuous intravenous infusion of [1-^{14}C]acetate is evaluated in a sheep and a steer and the efficacy of the method assessed by determining the effect of metabolizable energy (ME) intake on rates of lipogenesis in subcutaneous adipose tissue of young growing cattle.

Abbreviations: LW, live-weight; ME, metabolizable energy; R_{lipid}, rate of incorporation of acetate into lipid; SRA, specific radioactivity.

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Materials and methods

Animals, diets and experimental designs

Expt 1. To evaluate the technique, one cross-bred wether (56 kg final live weight (LW)) and one Hereford × Friesian steer (322 kg final LW) were used. Both animals were fed a compound feed to appetite and had free access to water and a mineral lick (Standard Wright Blocks; Frank Wright Ltd, Ashbourne, Derbys., UK) at all times. One week before the measurements of the rate of lipogenesis were made, the animals were placed in metabolism crates and introduced to an hourly feeding regime using automatic feeders.

Expt 2. The efficacy of the technique was determined by studying at the effect of ME intake on the rate of lipogenesis in fourteen Hereford × Friesian steers, initially 12 weeks of age. The steers were individually penned and had free access to water and a mineral lick (Standard Wright Blocks; Frank Wright Ltd) at all times. The steers were fed grass that had been artificially dried and pelleted, at one of seven ME intake levels (1·1, 1·2, 1·3, 1·4, 1·5, 1·8 and 2·0 (*ad libitum*) × ME requirement for maintenance) to which they were randomly allocated, such that there were two steers per intake level. Animals fed at the 2·0 × ME intake level were actually fed to appetite (fed 10 % in excess of the previous day's refusals), with this intake level representing the average *ad libitum* intake over the experimental period. Dietary feeding levels were calculated based on the animals, ME requirements for maintenance, which were calculated using the equation:

$$\text{ME for maintenance (MJ/d)} = \frac{(\text{LW} \times 0{\cdot}061) + 5{\cdot}67}{k_m},$$

where k_m is the efficiency with which ME is used for maintenance and was assumed to be 0·72 (Agriculture and Food Research Council, 1993).

The steers were introduced to their diets over a period of 7 d. At 13 weeks of age (mean LW 94·2 (SE 1·5) kg), the experimental period began, and lasted for 12–14 weeks. During the first 10–12 weeks of the experimental period, steers were weighed twice weekly and their dietary intakes adjusted accordingly. Diets were fed in two equal meals at 09.00 and 17.00 hours. Feed refusals were collected at 08.30 hours and weighed.

One week before the measurements of the rate of lipogenesis were made, the animals were placed in metabolism crates and introduced to an hourly feeding regimen using automatic feeders.

Preparation of infusate

The [1-^{14}C]acetate infusate was prepared on the day of infusion. [1-^{14}C]Acetate (2·2 GBq/mmol; ICN Pharmaceuticals Ltd, Thame, Oxon., UK) was added to sterile, pyrogen-free saline bags (0·15 M-NaCl; Baxter Healthcare Ltd, Thetford, Norfolk, UK) to give 0·5 kBq [1-^{14}C]acetate/kg LW per ml.

Experimental procedure

Two days before the measurement of the rate of lipogenesis, two indwelling sterile-polyurethane cannulas (1·2 mm i.d.; British Viggo, Swindon, Wilts., UK) were inserted into both jugular veins, one for the infusion of metabolic tracers (15 cm length, left cannula) and one for blood sampling (10 cm length, right cannula). The cannulas were kept patent by daily flushing with 0·13 M-tri-sodium citrate (dihydrate) in sterile saline (0·15 M-NaCl).

Venous blood samples (10 ml) were taken at 30 min intervals for a period of 90 min before the start of the 6 h continuous intravenous infusion of [1-^{14}C]acetate for the determination of background ^{14}C levels. The infusate was administered by peristaltic pump (Minipuls 2; Gilson, Villiers Le Bel, France) at a rate of about 2 ml/min, the exact rate being determined by gravimetric difference. Blood samples (10 ml) were then taken at 5, 10, 15, 20, 25, 30, 40, 50 and 60 min after commencement of the infusion and at 20 min intervals thereafter until the end of the infusion for the measurement of plasma acetate specific radioactivity (SRA).

Blood samples were collected into ice-chilled centrifuge tubes containing heparin (25 units/ml blood). Blood samples were gently mixed by inversion and then centrifuged at 1000 *g* for 15 min to isolate the plasma which was stored at −20°C.

In Expt 1, adipose tissue biopsies (200–300 mg) were taken at 60, 160, and 260 min after commencement of the infusion from the tail region around the coccygeal vertebra under local anaesthetic (Lignavet, 20 g lignocaine hydrochloride/l; C-VET Ltd, Bury St Edmunds, Suffolk, UK). At the end of the 6 h infusion period, but before the infusion was terminated, the animals were given a lethal dose of pentobarbitone (Euthesate, 200 g pentobarbitone sodium/l; Williams Francis Veterinary, Crawley, West Sussex, UK) and a final adipose tissue sample immediately taken from the same region as the biopsies.

In Expt 2, a single adipose tissue sample was taken at the end of the 6 h infusion. At the end of the infusion, but before the infusion was terminated, the steers were killed with a lethal dose of pentobarbitone and exanguinated. The infusion was then immediately terminated and a subcutaneous adipose tissue sample (5 g) taken from tail region around the coccygeal vertebra.

The adipose tissue samples were quickly washed and chopped in ice-cold saline (0·15 M-NaCl), and then weighed (about 500 mg adipose tissue (Expt 2)) into 50 ml Falcon tubes containing 5 ml Doles Reagent (propan-2-ol–heptane–0·5 M-H_2SO_4 (40:10:1, by vol.); Dole & Meinertz, 1960). The samples were then immediately homogenized for 30 s after which they were placed in a freezer (−20°C) until analysed.

Analytical methods and calculations

Dietary analysis. The chemical composition of the dried grass diet (Table 1) was determined as described by England & Gill (1983). All values were corrected for DM determined by oven drying at 100°C for 24 h.

Plasma acetate specific radioactivity. The SRA of plasma acetate was determined using a modified version of the method described by Persson *et al.* (1991). Plasma samples (1·0 ml) and calibration standards (1·0 ml control plasma spiked with 50 μl appropriate working sodium

Table 1. Chemical composition (g/kg DM) of the dried grass diet used in Expt 2

DM (g/kg fresh diet)	916·8
Organic matter	914·9
Total N	28·4
Soluble N (g/kg total N)	187·7
Insoluble N (g/kg total N)	810·9
Ash	85·1
Neutral detergent fibre	536·7
Acid detergent fibre	270·9
MADF	271
Acid detergent fibre N	2·7
Starch	25·1
Water-soluble carbohydrate	149·6
Gross energy (MJ/kg DM)	18·7
Metabolizable energy (MJ/kg DM)*	10·83

MADF, modified acid detergent fibre.
* Estimated from MADF.

acetate calibration standard solution) over the range 0·3–1·7 mM-sodium acetate were pipetted into 12 ml screw-capped Pyrex culture tubes (Aldrich Chemical Co., Gillingham, Dorset, UK). To all tubes was then added internal standard (100 μl; 250 Bq [2-^{3}H]sodium acetate (ICN Pharmaceuticals Ltd)/ml 2 mM-Na_2CO_3). After vortexing, samples were acidified by the addition of 100 μl 1 M-HCl and the samples again vortexed. Diethyl ether (5 ml) was then added and the tubes immediately capped. The samples were placed on an automatic shaker for 20 min after which they were centrifuged (1000 ***g*** for 10 min). The ether layer, containing the acetate, was then transferred to another screw-capped culture tube containing 1 ml 2 mM-NaOH, and immediately capped. The ether extraction was repeated and the ether layers pooled. The tube containing the pooled ether layers plus 2 mM-NaOH was shaken for 5 min and then centrifuged (1000 ***g*** for 5 min). The ether layer was aspirated off and discarded. The remaining sample containing the sodium acetate was taken to dryness under a stream of N_2 and then derivatised to the phenacyl derivative.

To the dried samples, 200 μl mixed solution of α-bromoacetophenone (40 mM; Aldrich Chemical Co.) and 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane) (80 mM; Aldrich Chemical Co.) in acetone was added. The tubes were capped and vortexed, then sonicated in an ultrasonic water bath (Ultrawave U300; Radleys, Saffron Waldon, Essex, UK) for 30 min before being placed in an oven at 100°C for 15 min. After cooling for 10 min, 100 μl 150 mM-propionic acid in acetone was added to each tube to react with the remaining excess of α-bromoacetophenone. The tubes were vortexed and then heated for a further 5 min in an oven at 100°C. After cooling, the tubes were taken to dryness under a stream of N_2. The samples were resuspended in 260 μl acetonitrile (200 ml/l water), transferred to Eppendorf tubes (0·5 ml) and stored at 4°C until analysed by HPLC.

Using a HP1050 Series (pump and variable wavelength detector) HPLC (Hewlett Packard Ltd, Stockport, Ches., UK), 200 μl sample was injected onto a Spherisorb ODS-2 column (5 μm, 250 × 4 mm; Hewlett Packard Ltd) maintained at 50°C with a water-jacket. The mobile phase was acetonitrile (200 ml/l water) and the flow rate 1·2 ml/min. Detection was at 320 nm. Data was processed with a HP3396 Series II integrator (Hewlett Packard Ltd). Acetate eluted at about 12·5 min and was collected as a single fraction over a 2·5 min period (3 ml) using a fraction collector (Frac-100; Pharmacia Fine Chemicals, Uppsala, Sweden).

The collected acetate fractions were transferred to glass scintillation vials in which their volume was reduced to approximately 1 ml under a stream of air. Liquid scintillant (10 ml; Emulsifier Scintillant Plus; Packard Instrument B.V. – Chemical Operations, Groningen, The Netherlands) was then added and the [1-^{14}C]acetate and [2-^{3}H]acetate counted using a Packard 1900CA Tri-Carb Liquid Scintillation Analyser (Packard Instrument Co., Meriden, Connecticut USA) in dual label mode to discriminate between the ^{14}C and ^{3}H label.

Acetate peak areas and ^{14}C-counts were corrected for recovery (^{3}H added/^{3}H measured). Plasma acetate concentrations were determined using the corrected sample peak areas by interpolation from a standard curve of corrected acetate standard peak area *v.* standard acetate concentration and these values used for the calculation of plasma acetate SRA. The inter-assay CV for acetate concentration was 7·7 % (*n* 24).

Total lipid extraction from adipose tissue and total lipid specific radioactivity determination. Total lipid was extracted from adipose tissue using the method of Dole & Meinertz (1960). The amount of [1-^{14}C]acetate incorporated into the total lipid was quantified by scintillation counting. Total lipid SRA was expressed as Bq/g lipid.

Calculation of the rate of lipogenesis. The rate of acetate incorporation into adipose tissue lipid (R_{lipid}) was calculated from the increase in accumulation of ^{14}C in adipose lipid over time. It was assumed that all radioactivity in the adipose tissue lipid originated from the [1-^{14}C]acetate in the blood, and therefore that:

$$R_{lipid} = \frac{\Delta SRA_{lipid}}{\sum_{t_1}^{t_2} SRA_{acetate}},$$

where R_{lipid} is the rate of acetate incorporation into adipose tissue lipid (μg acetate/g lipid per min), ΔSRA_{lipid} is the change in the adipose tissue lipid SRA (Bq/g lipid) between t_1 and t_2, and $\sum_{t_1}^{t_2} SRA_{acetate}$ is the cumulative area (Bq × min of infusion/μg acetate) under the plasma acetate SRA *v.* time (min) curve from the start of the infusion (t_1) until the time the adipose tissue sample was taken (t_2).

Statistics

In Expt 1, the relationship between [1-^{14}C]acetate incorporation into lipid and the time after the start of the [1-^{14}C]acetate infusion, and in Expt 2 the response in R_{lipid} to ME intake, were described by linear regression analysis.

All animal work described was carried out under licence of the Animals (Scientific Procedures) Act, 1986.

Table 2. Comparison of estimates of the rate of acetate incorporation into the total lipid of subcutaneous adipose tissue samples sequentially obtained by biopsy from a sheep during the continuous intravenous infusion of [1-^{14}C]acetate*

Time of biopsy (min)		ΔSRA_{lipid}	$\sum_{t_1}^{t_2} SRA_{acetate}$	R_{lipid}
t_1	t_2	(Bq/g lipid)†	(Bq × min of infusion/μg acetate)‡	(μg/min per g lipid)§
0	60	33·98	7·60	4·5
0	160	101·19	16·46	6·1
0	260	141·25	28·71	4·9
0	360	185·94	40·32	4·6

* For details of diets and procedures, see Table 1 and pp. 38–39.
† Change in specific radioactivity of total lipid (SRA_{lipid}) extracted from subcutaneous adipose tissue between t_1 and t_2.
‡ Cumulative area under the plasma acetate specific radioactivity ($SRA_{acetate}$) *v.* time curve between t_1 and t_2.
§ Rate of acetate incorporation into total lipid (R_{lipid}).

Results

Expt 1

An *in vivo* method for the measurement of the rate of lipogenesis in ruminants using a continuous intravenous infusion of [1-^{14}C]acetate was assessed in a wether and a steer. Tables 2 and 3 show the cumulative increases in acetate SRA in plasma and in adipose tissue lipid with time and the calculated rates of lipogenesis at each time point in the sheep and steer respectively. The R_{lipid} values calculated for the sheep were approximately 2-fold lower than those recorded for the steer (4·6 *v.* 10·3 μg/min per g lipid respectively). Figs 1 and 2 illustrate plasma acetate SRA and adipose tissue lipid SRA *v.* time in the sheep and the steer respectively. The continuous intravenous infusion of [1-^{14}C]acetate resulted in a rapid increase in plasma acetate SRA reaching a plateau within 45 min in both the sheep and steer. Isotopic equilibrium had therefore been reached by the time of the first adipose tissue biopsy 60 min into the infusion.

The rate of acetate incorporation into total adipose tissue lipid (R_{lipid}) was constant. This was reflected by the linear increase in the SRA of adipose tissue lipid in both the sheep (r^2 0·99) and steer (r^2 0·99), and the close agreement between R_{lipid} values calculated from the adipose tissue samples obtained by biopsy at the different time points (Tables 2 and 3). Based on these results, it was decided that in Expt 2 only a single adipose tissue sample taken at the end of the [1-^{14}C]acetate infusion was required for the determination of the rate of acetate incorporation into total lipid, provided that the same blood sampling schedule was followed to assure that the area under the plasma acetate SRA curve could be accurately determined.

Expt 2

The effect of ME intake on *in vivo* rates of lipogenesis in the subcutaneous adipose tissue of steers was determined using the method evaluated in Expt 1 (Fig. 3). There was a significant ($P < 0·001$) linear effect of ME intake on the rate of acetate incorporation into the total lipid of subcutaneous adipose tissue. R_{lipid} values increased from 0·075 μg acetate/min per g lipid at a ME intake level of 1·163 × maintenance requirement, to 6·567 μg acetate/min per g lipid at the highest ME intake level.

Discussion

Continuous intravenous infusions of [U-^{14}C]glucose have been used to measure *in vivo* rates of triacylglycerol–glycerol synthesis in sheep and *in vivo* rates of lipogenesis in pigs by Davey (1986) and Dunshea *et al.* (1992) respectively. Here, a technique for the measurement of *in vivo* rates of lipogenesis in ruminants is described and evaluated using a continuous intravenous infusion of [1-^{14}C]acetate. Acetate was used as the tracer since it is the principal lipogenic precursor in ruminants (Vernon, 1981). The principles on which this technique and those described by Davey (1986) and Dunshea *et al.* (1992) are based are the same as those that have been extensively used to obtain protein synthetic rates (Waterlow *et al.* 1978). That is, through the estimation of the area under the curve

Table 3. Comparison of estimates of the rate of acetate incorporation into the total lipid of subcutaneous adipose tissue samples sequentially obtained by biopsy from a steer during the continuous intravenous infusion of [1-^{14}C]acetate*

Time of biopsy (min)		ΔSRA_{lipid}	$\sum_{t_1}^{t_2} SRA_{acetate}$	R_{lipid}
t_1	t_2	(Bq/g lipid)†	(Bq × min of infusion/μg acetate)‡	(μg/min per g lipid)§
0	60	102·45	6·77	15·1
0	160	235·03	19·34	12·2
0	260	404·07	32·88	12·3
0	360	487·36	47·14	10·3

* For details of diets and procedures, see Table 1 and pp. 38–39.
† Change in specific radioactivity of total lipid (SRA_{lipid}) extracted from subcutaneous adipose tissue between t_1 and t_2.
‡ Cumulative area under the plasma acetate specific radioactivity ($SRA_{acetate}$) *v.* time curve between t_1 and t_2.
§ Rate of acetate incorporation into total lipid (R_{lipid}).

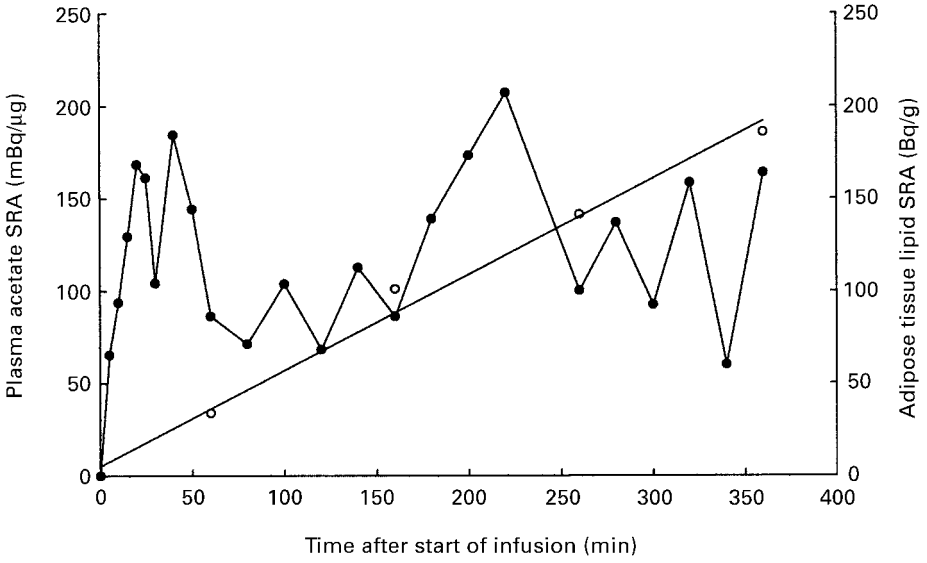

Fig. 1. Plasma acetate specific radioactivity (SRA) (●) and adipose tissue lipid SRA (○) during a continuous intravenous infusion of [1-^{14}C]acetate into a sheep. For details of diets and procedures, see Table 1 and pp. 38–39. The relationship between adipose tissue lipid SRA and time after the start of the infusion is described by the equation $y = 0{\cdot}52x + 5{\cdot}36$ (r^2 0·990, $P < 0{\cdot}001$).

depicting the precursor pool and coupling this with incorporation into tissue, so dynamic rates of synthesis can be calculated. These techniques are therefore dependent on isotope dilution and isotope incorporation methodology.

The isotope dilution technique is dependent on the subject being in a 'metabolic steady state' so that a plateau in plasma isotopic activity can be obtained, which indicates that a dynamic equilibrium exists with tracer entry rate into the plasma pool equalling disappearance rate. Dunshea *et al.* (1992) gave a priming dose of [U-^{14}C]glucose immediately before the start of the continuous intravenous infusion of [U-^{14}C]glucose in order to achieve more rapidly a plateau in isotopic activity, which, without the aid of a priming dose, can take 2–4 h to reach (Davey, 1986). In the case of acetate, a priming dose was not considered necessary since the plasma acetate pool has a rapid rate of turnover (0·1–0·2 mmol/min per kg$^{0.75}$; Cronjé *et al.* 1991). In both the wether and the steer, isotopic equilibrium in the plasma [1-^{14}C]acetate was achieved within 45 min (Figs 1 and 2).

The method of measuring lipogenesis *in vivo* described here makes the assumption that the precursor pool for lipogenesis (acetyl-CoA) has the same isotopic activity as the plasma acetate pool. This is unlikely to be strictly true,

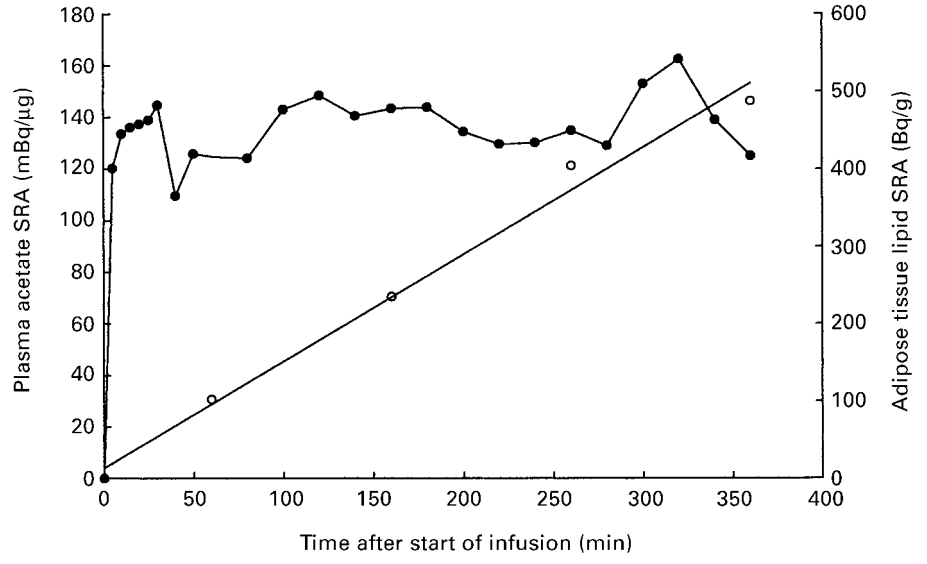

Fig. 2. Plasma acetate specific radioactivity (SRA) (●) and adipose tissue lipid SRA (○) during a continuous intravenous infusion of [1-^{14}C]acetate into a steer. For details of diets and procedures, see Table 1 and pp. 38–39. The relationship between adipose tissue lipid SRA and time after the start of the infusion is described by the equation: $y = 1{\cdot}38x + 13{\cdot}71$ (r^2 0·989, $P < 0{\cdot}001$).

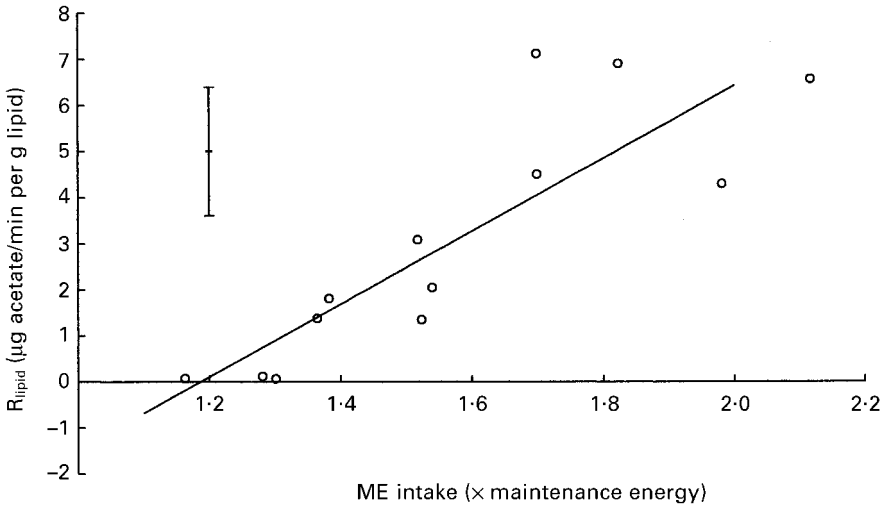

Fig. 3. Rates of acetate incorporation (R_{lipid}) into the total lipid of subcutaneous adipose tissue of individual steers fed dried grass at a range of metabolizable energy (ME) intakes. For details of diets and procedures, see Table 1 and pp. 38–39. The relationship between R_{lipid} and ME intake is described by the equation: $y = 7{\cdot}90x - 9{\cdot}37$ (r^2 0·738, $P < 0{\cdot}001$). The error bar is the residual SD (11 d.f.).

since the primary pool (pool into which the tracer is infused) will always have a higher isotopic activity than a secondary pool, the difference between which could result in an underestimation of rates of lipogenesis. However, the difference in activities between the intra-adipocyte acetyl-CoA pool and the plasma acetate pool are thought likely to be relatively small. This is because acetate crosses membranes by passive diffusion down a concentration gradient (Madsen, 1983), and since conversion of acetate to acetyl-CoA by acetyl-CoA synthetase is concentration dependent (Vernon, 1981).

An objective of Expt 1 was to demonstrate that acetate incorporation into lipid was linear with time in both the sheep and the steer. The linear increase in the SRA of adipose tissue lipid in both the sheep and steer (Figs 1 and 2 respectively), and the close agreement between individual biopsy R_{lipid} values (Tables 2 and 3) was proof of this. Even the large amount of variation associated with the plasma acetate SRA plateau of the sheep compared with that of the steer (7·38 (SD 2·664) kBq/mmol acetate *v.* 8·18 (SD 0·754) kBq/mmol acetate respectively) did not prevent the linear incorporation of [1-^{14}C]acetate into adipose tissue lipid, illustrating how, provided the area under the plasma acetate SRA curve can be accurately measured, the relationship between R_{lipid} and $\sum_{t_1}^{t_2} SRA_{acetate}$ remains the same, providing R_{lipid} remains constant. Based on these data it was decided that only a single adipose tissue sample need be taken during a continuous intravenous infusion of [1-^{14}C]acetate to the measure the rates of [1-^{14}C]acetate incorporation into adipose tissue lipid, which was considered to be important in order to minimize stress to the animal, and thus ensure that the animal remains in a 'metabolic steady state' throughout the course of the measurement period. This approach was successfully adopted in Expt 2, with adipose tissue samples being collected from the subcutaneous adipose tissue depot at the end of the [1-^{14}C]acetate infusion immediately after the slaughter of the animals.

In both Expts 1 and 2, subcutaneous adipose tissue was sampled from the tail region around the coccygeal vertebra, which, in the case of Expt 1, was for reasons of practicality and accessibility. It is important to realize that the R_{lipid} values recorded are not only depot-specific, but also within-depot site-specific (Pond, 1999). The various fat depots do not develop at the same time, or at the same rate. Generally the subcutaneous adipose tissue depot develops after the intra-abdominal depots and before the intramuscular depot (Vernon, 1992; Annison, 1993). In ruminants, lipogenesis is higher in subcutaneous than in abdominal fat (Vernon, 1981). Within the subcutaneous adipose tissue depot, the anterior shoulder and interscapular depots have a greater metabolic activity than the posterior superficial groin depot (Pond, 1992). It is therefore essential that inter-animal comparisons in R_{lipid} be made between adipose tissue samples taken from the same depot and from the same region within that depot if meaningful comparisons are to be made. The method described for measuring rates of lipogenesis *in vivo* is not limited to measuring R_{lipid} values in subcutaneous adipose tissue. The technique as applied in Expt 2, i.e. with the slaughter of the animal at the end of the infusion period, potentially enables any adipose tissue depot to be sampled. This makes the technique all the more useful in that it enables relative rates of lipogenesis in the different depots to the measured and enables a more complete assessment of the lipogenic state of the animal.

The measurements yielded by the method are fractional synthetic rates, not absolute synthetic rates. The fractional synthetic rates enable rates of metabolic activity to be compared between adipose tissues from different depots of the same animal and, as was the case in Expts 1 and 2, from the same depots of different animals. Absolute synthetic rates are dependent upon both depot size and fractional synthetic rate. The method can be used to calculate absolute synthetic rates in easily dissectable adipose tissue depots such as the perirenal depot, the masses of which can be

measured, or it may be possible to couple the method to techniques such as ultrasound which can estimate, for example backfat mass.

The rate of lipogenesis measured for the steer in Expt 1 was 10·3 μg acetate/min per g lipid (Table 3). This was 2–4-fold higher than *in vitro* rates of lipogenesis measured in subcutaneous adipose tissue explants of steers (2·67–5·3 μg acetate/min per g lipid; Dawson *et al.* 1999), measurements made in the same laboratory as the *in vivo* measurements were made. Obviously, allowances must be made for differences in diet (composition and intake), sex, breed and age of the animals, since all have an effect on the rate of lipogenesis (Vernon, 1981). Dunshea *et al.* (1992) similarly reported that *in vivo* rates of lipogenesis in pigs were typically 3-fold higher than those measured *in vitro*. *In vitro* rates of lipogenesis are considered to underestimate rates of lipogenesis, since adipose tissue explants are in a net degradative state (Mersmann, 1986). Furthermore, rates of non-esterified fatty acid re-esterification tend to be high as a result of limited albumin, and there is an accumulation of adenosine in the incubating medium, all of which have influences on the rate of lipogenesis. In contrast, the rate of lipogenesis measured for the sheep in Expt 1 (4·6 μg acetate/min per g lipid; Table 2) fell within the upper range typically reported from *in vitro* incubations of subcutaneous adipose tissue (0·68–5·6 μg acetate/min per g lipid; Vernon, 1981). The rate of lipogenesis in the sheep may have been depressed as a consequence of stress (Vernon, 1992) since it was less used to being handled than the steer was.

Rates of lipogenesis were greater in the subcutaneous adipose tissue of the steer than the sheep (10·3 *v.* 4·6 μg acetate/min per g lipid respectively). This could have been a species difference and/or a difference in mature age between the wether and the steer. Ingle *et al.* (1972*a*) have shown that calves and market steers generally have greater rates of acetate incorporation into adipose tissue than do lambs and mature sheep, and Vernon (1981) found that there is a decline in the rate of lipogenesis with maturity despite the increased rate of lipid deposition. The sheep used (56 kg LW) was considered to be mature, while the steer (322 kg LW) was not.

In Expt 2, the technique for the measurement of the rate of lipogenesis *in vivo* using a continuous intravenous infusion of [1-^{14}C]acetate was used to investigate the effect of ME intake on the rate of lipogenesis in subcutaneous adipose tissue of young growing steers. The significant ($P < 0{\cdot}001$) linear effect of ME intake on the rate of lipogenesis in subcutaneous adipose tissue was as expected and in agreement with the understanding that fat deposition increases with increasing energy intake in excess of maintenance energy intake (Berg & Walters, 1983). Mills *et al.* (1989) have reported rates of lipogenesis in subcutaneous adipose tissue explants ranging from 1·02 to 5·52 μg acetate/min per g lipid for steers fed to gain 0–1·5 kg LW/d (feed intake *ad libitum*) respectively. Similarly, Smith *et al.* (1992) measured *in vitro* rates of lipogenesis in subcutaneous adipose tissue explants ranging from 0·014 to 3·710 μg acetate/min per g lipid in ovariectomised heifers fed a diet over a range of ME intakes ranging from 0·76 to 2·05 × ME requirement for maintenance respectively.

Table 4. Balance sheet for the fate of acetate in the sheep and steer used in Expt 1*

Variable	mg/min	
	Sheep	Steer
Acetate entry rate†	372	2967
Adipose tissue lipid synthesis‡	64	591
Non-adipose-tissue lipid synthesis utilization§	308	2376

* For details of diets and procedures, see Table 1 and pp. 38–39.
† Calculated from the acetate kinetic data generated from Expt 1.
‡ Calculated assuming: for the sheep an empty-body weight of 50·6 kg and an empty-body fat content of 275 g/kg, and for the steer an empty-body weight of 291·4 kg and an empty-body fat content of 197 g/kg (Agriculture Research Council, 1980).
§ Derived from the difference between acetate entry rate and rate of adipose tissue lipid synthesis.

A useful additional measurement that can be made at the same time as measuring R_{lipid} is acetate entry rate, which can be calculated from the known infusion rate of [1-^{14}C]acetate and the determined plasma acetate SRA at plateau. In Expt 1, the acetate entry rate for the sheep was 0·303 mmol/min per $kg^{0{\cdot}75}$ and for the steer was 0·650 mmol/min per $kg^{0{\cdot}75}$ (results not shown). This information can be used to construct a balance sheet (Table 4) for the fate of acetate entering the plasma pool providing the fat content of the animal is known and assuming a whole-body R_{lipid} value. It was calculated that lipogenesis accounts for approximately 20 % acetate leaving the plasma pool in both the sheep and the steer. Ideally, the fat content of the animal would be determined through carcass composition analysis. As already discussed, R_{lipid} values are depot-specific, so absolute rates of acetate incorporation into lipid for each adipose tissue depot, should be measured in order to accommodate the inter-depot differences in R_{lipid}.

Based on the results from Expts 1 and 2, it is concluded that the technique for the measurement of lipogenesis in ruminants by means of a continuous intravenous infusion of [1-^{14}C]acetate is effective. The technique should prove to be a valuable tool for both the quantitative and comparative study of lipid metabolism in ruminant animals.

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