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Metabolism of *R*- and *S*-1,3-butanediol in perfused livers from meal-fed and starved rats

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The metabolism of millimolar concentrations of *R*- or *S*-1,3-butanediol has been studied in perfused livers from fed and starved rats. Protocols were designed to measure in the same experiment (i) uptake of the diol, (ii) the contribution of the diol to ketogenesis, (iii) the contribution of the diol to total fatty acid plus sterol synthesis, and (iv) conversion of *S*-1,3-butanediol into *S*-3-hydroxybutyrate. Our data show that *R*- and *S*-1,3-butanediol are taken up by the liver at the same rate. Most of the metabolism of *R*-1,3-butanediol is accounted for by conversion to the physiological ketone bodies *R*-3-hydroxybutyrate and acetoacetate. Only 29–38% of *S*-1,3-butanediol uptake is accounted for by conversion into physiological ketone bodies. The balance of *S*-1,3-butanediol metabolism is conversion to *S*-3-hydroxybutyrate, lipids and CO_2.

INTRODUCTION

RS-1,3-Butanediol has been investigated as a carbon source for animal [1–6] and human [7,8] nutrition. It has also been proposed as a therapeutic agent for the ethanol withdrawal syndrome [9]. The *S*- (but not the *R*-) enantiomer, when added to the diet of alloxan-diabetic rats, appears to normalize blood glucose [10]. This raises the possibility of using *S*-1,3-butanediol as part of the nutritional therapy of diabetes.

RS-1,3-Butanediol is oxidized in the liver to *RS*-3-hydroxybutyrate (*RS*-BHB) by alcohol and aldehyde dehydrogenase. *R*-BHB (note that the physiological enantiomer *R*-BHB is designated as D-BHB in the recent clinical literature. In early publications [11,12] and in the Merck Index, it is called L-BHB) is a physiological ketone body which is in oxidoreduction equilibrium with acetoacetate (AcAc) via *R*-BHB dehydrogenase. *S*-BHB is not a natural compound [13]. We have shown [14] that *S*-BHB is metabolized in the liver via mitochondrial activation to *S*-BHB-CoA, one of the final intermediates of fatty acid β-oxidation. The subsequent fate of *S*-BHB in the liver is incorporation into physiological ketone bodies (*R*-BHB + AcAc), lipids and CO_2.

The goal of the present study was to characterize the metabolism of the two 1,3-butanediol enantiomers in the liver, the site of the initial steps of their metabolism.

MATERIALS AND METHODS

Chemicals

Enzymes and coenzymes were purchased from Boehringer–Mannheim Canada Ltd. (Dorval, Quebec, Canada). Sodium *RS*-BHB, $LiAlH_4$, $NaBH_4$ and quinine base were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethyl-AcAc, sulphosalicyclic acid, tetrahydrofuran and diethyl ether were bought from Fisher Scientific (Montreal, Quebec, Canada). *R*- and *RS*-[3-^{14}C]BHB (33 mCi/mmol), [2-^{14}C]acetone, $NaH^{14}CO_3$, internal standards of [^{3}H]- and [^{14}C]-toluene were purchased from New England Nuclear. ^3H_2O (5 Ci/g) was obtained from Amersham. Ethyl-[3,4-$^{13}C_2$]AcAc, deuterium oxide, sodium deuteroxide (40% NaO^2H in ^2H_2O) and NaB^2H_4, all with molar percent enrichment (MPE) > 99%, were obtained from Merck Sharp and Dohme Isotopes (Pointe-Claire, Quebec, Canada). The purity of all ^{13}C-labelled tracers was confirmed as described previously [15]. *N*-Methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide was obtained from Regis Chemical Company (Morton Grove, IL, U.S.A.).

By enzymic assay with *R*-BHB dehydrogenase, we tested the *R*- and *S*-BHB sold by Aldrich as 98% optically pure. We found for each compound 10% contamination with the opposite enantiomer. Since *R*- and *S*-1,3-butanediol are probably synthesized from *R*- and *S*-BHB or from precursors of these, we suspected similar cross-contamination of the 1,3-butanediol enantiomers. Thus we prepared the unlabelled and ^{14}C-labelled *R*- and *S*-BHB used for synthesis of millimolar quantities of *R*- and *S*-[3-^{14}C]1,3-butanediol.

Unlabelled and [3,4-$^{13}C_2$]AcAc were prepared by hydrolysis of the corresponding ethyl esters [15]. Unlabelled *R*-BHB and *R*-[3-^{14}C]BHB were prepared by enzymic reduction of the corresponding AcAc [16]. Unlabelled *S*-BHB was prepared from the *RS*- mixture by the method of McCann [17]. *S*-[3-^{14}C]BHB was prepared from the *RS*- mixture by enzymic conversion of the *R*-[3-^{14}C]BHB moiety into [3-^{14}C]AcAc [14]. After the reaction, *S*-[3-^{14}C]BHB and [3-^{14}C]AcAc were separated by chromatography on AG1-X8-C1 resin [18]. *R*- and *S*-[3-^{14}C]1,3-butanediol were synthesized by reduction of the corresponding [3-^{14}C]BHB acid with $LiAlH_4$ suspended in tetrahydrofuran [20]. The internal standard of *RS*-[3,4-$^{13}C_2$]1,3-butanediol was synthesized by $LiAl^2H_4$ reduction of ethyl [3,4-$^{13}C_2$]AcAc [20]. The chemical purity of *R*- and *S*-[3-^{14}C]1,3-butanediol was checked by: (i) h.p.l.c. on an Aminex HPX-87 column developed with 0.005 M-H_2SO_4, and (ii) mass spectrometry of bis-*tert*-butyldimethylsilyl (bis-TBDMS) derivative as described below. Specific radio-

Abbreviations used: AcAc, acetoacetate; AcAc-CoA, acetoacetyl-CoA; *RS*-BHB, *RS*-3-hydroxybutyrate; [$^2H_6$]BHB, *RS*-3-hydroxy[2,2,3,4,4,4-$^2H_6$]butyrate; g.c.–m.s., gas chromatography–mass spectrometry; MPE, molar percent enrichment; SA, specific radioactivity; TBDMS, *t*-butyldimethylsilyl.

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activities of *R*- and *S*-[3-^{14}C]1,3-butanediol were 11920 and 26650 d.p.m./μmol respectively.

Animals

Male Sprague–Dawley rats (Charles River) were kept at 22–24 °C and fed on Charles River Laboratory chow from 07:00 to 10:00 h. The animals were used during the third week of the feeding schedule, when they were in the weight range 210–280 g. Experiments were started between 10:00 and 11:00 h. This period corresponds to the maximum rate of fatty acid and sterol synthesis under these conditions of feeding [22]. Rats designated as 'starved' received their last meal 48 h before the experiment.

Liver perfusions

Livers were perfused [23] with 210 ml of recirculating Krebs–Ringer bicarbonate buffer containing 4% dialysed BSA (fraction V, fatty-acid-poor; Miles Scientific), and glucose (15 or 4 mM in perfusions of livers from fed or starved rats respectively). After 30 min of equilibration, *R*- or *S*-[3-^{14}C]1,3-butanediol (fed, 5 mM; starved, 7.5 mM), 80 μmol of [3,4-$^{13}C_2$]AcAc and 25 mCi of ^3H_2O were added to the perfusate. At 120 min, livers were freeze-clamped and kept at −80 °C until analysis.

Analytical procedures

Samples of perfusate were assayed for concentrations [24,25] and specific radioactivity (SA) [18] of *R*-BHB and AcAc. AcAc isolated by column chromatography [18] was degraded [26] to acetone (from C-2 to C-4 of AcAc) and CO_2 (C-1 of AcAc), which were trapped in hydrazine–lactate and NaOH respectively before counting of radioactivity in Hionic-Fluor. Recovery standards of [3-^{14}C]AcAc, [2-^{14}C]acetone and Na$H^{14}CO_3$ were run in parallel with the samples.

Other samples (1 ml) of perfusate were treated with 50 μl of 1 M-NaB^2H_4/0.1 M-NaOH to reduce AcAc and [3,4-$^{13}C_2$]AcAc to the corresponding deuteriated BHB species [15]. The MPE of perfusate AcAc and *R*-BHB and the concentration of 1,3-butanediol were measured [15,20] by g.c.–m.s.

$^{14}CO_2$ production

At the end of the experiment, $^{14}CO_2$ from the sealed gas phase was trapped by adding 0.3 ml of 10 M-NaOH to the recirculating perfusate [23]. Samples of alkaline perfusate (10 ml) were adjusted to 50 mM-$NaBH_4$ before acidification, to (i) decrease the volatility of [2-^{14}C]acetone by conversion into [2-^{14}C]propan-2-ol, and (ii) convert labile AcAc into stable *RS*-BHB. This procedure avoids the artifactual production of $^{14}CO_2$ by decarboxylation of [1,3-^{14}C]AcAc generated during perfusions with *S*-[3-^{14}C]1,3-butanediol. CO_2 released by acidification was trapped in 2 ml of NaOH (2 M). To avoid possible contamination of $^{14}CO_2$ by [3-^{14}C]1,3-butanediol, [2-^{14}C]propan-2-ol or ^3H_2O, the trapping solution was diluted with 1 ml of a solution containing 10 mM-1,3-butanediol, 10 mM-propan-2-ol and 25 mM-NaOH. Then, 0.2 ml of barium acetate (1.5 M) was added to precipitate $^{14}CO_2$ as barium carbonate. The precipitate was washed three times with 0.5 ml of 10 mM-1,3-butanediol, 10 mM-propan-2-ol and 1.0 M-barium acetate. $^{14}CO_2$ was evolved again by acidification and trapped in 0.35 ml of NaOH (3 M) before counting radioactivity in Hionic-Fluor. Recovery standards of Na$H^{14}CO_3$ were run in parallel with the samples.

The total rates of fatty acid and 3β-hydroxysterol synthesis in the liver and the contribution of [3-^{14}C]1,3-butanediol to lipogenesis were calculated from the incorporation of ^{3}H and ^{14}C respectively [18]. The sterols were recrystallized following hydrolysis of the digitonide [27].

All amounts of radioactivity measured by liquid scintillation counting were converted to d.p.m. by internal standardization with [^{3}H]- or [^{14}C]-toluene, or both sequentially.

THEORY

Mass spectrometric data

Areas under each fragmentogram were determined by computer integration and were corrected for naturally occurring heavy isotopes and light isotopic impurities as described previously [15]. The corrected area for each isotopomer of BHB is designated as a_n, where n corresponds to the m/z ratio of the ion monitored. Fragments monitored at m/z 275 and 277 correspond to unlabelled BHB and [3,4-$^{13}C_2$]BHB respectively. Fragments monitored at m/z 276 and 278 correspond to [3-$^2H_1$]BHB and [3-$^2H_1$, 3,4-$^{13}C_2$]BHB, derived from NaB^2H_4 reduction of AcAc and [3,4-$^{13}C_2$]AcAc respectively.

Calculations of the MPE of *R*-[3,4-$^{13}C_2$]BHB in control perfusions and perfusions with *R*-1,3-butanediol are different from those in perfusions with *S*-1,3-butanediol in which *S*-BHB is generated. In control and *R*-1,3-butanediol perfusions, MPEs of *R*-[$^{13}C_2$]BHB and of [$^{13}C_2$]AcAc were calculated as:

$$\text{MPE of } R\text{-}[^{13}C_2]\text{BHB } (\%) = \frac{a_{277} \times 100}{a_{275} + a_{277}} \quad (1)$$

and

$$\text{MPE of } [^{13}C_2]\text{AcAc } (\%) = \frac{a_{278} \times 100}{a_{276} + a_{278}} \quad (2)$$

Note that, because of the treatment of samples with NaB^2H_4, perfusate AcAc and [3,4-$^{13}C_2$]AcAc are assayed by g.c.–m.s. as *RS*-[3-$^2H_1$]BHB and *RS*-[3-$^2H_1$,3,4-$^{13}C_2$]BHB respectively.

In perfusions with *S*-1,3-butanediol, the area at m/z 275 includes signals from both *S*-BHB and unlabelled *R*-BHB. The component of the area at m/z 275 corresponding to unlabelled *R*-BHB ($a_{R\text{-BHB}}$) was calculated using the ratio $[R\text{-BHB}]_{enz.}/[\text{AcAc}]_{enz.}$ determined enzymically:

$$a_{R\text{-BHB}} = (a_{276} + a_{278}) \times \frac{[R\text{-BHB}]_{enz.}}{[\text{AcAc}]_{enz.}} - a_{277} \quad (3)$$

where $(a_{276} + a_{278})$ represents the sum of areas corresponding to AcAc and [3,4-$^{13}C_2$]AcAc, and a_{277} represents the area corresponding to *R*-[3,4-$^{13}C_2$]BHB. Note that the enzymic assays of *R*-BHB and AcAc yield the total concentrations of unlabelled + labelled isotopomers.

We have previously shown [20] that the concentration of *S*-BHB can be calculated by the difference between (i) the concentration of *R*- + *S*-BHB assayed by g.c.–m.s., and (ii) the concentration of *R*-BHB assayed enzymically. Since the concentration of *R*-BHB measured enzymically comprises both *R*-BHB and *R*-[$^{13}C_2$]BHB, the concentration of *S*-BHB ([*S*-BHB]) was calculated as:

$$[S\text{-BHB}] = \frac{(a_{275} - a_{R\text{-BHB}}) \times [\text{AcAc}]_{enz.}}{(a_{276} + a_{278})} \quad (4)$$

where a_{275} is the area corresponding to *R*-BHB + *S*-BHB, $a_{R\text{-BHB}}$ is taken from eqn. (3), and $(a_{276} + a_{278})$ is the sum of the areas corresponding to AcAc and [$^{13}C_2$]AcAc. The ratio $[\text{AcAc}]_{enz.}/(a_{276} + a_{278})$ allows us to convert the areas corresponding to *S*-BHB at m/z 275 to the *S*-BHB concentration.

Ketone body production

Endemann *et al.* [18] have calculated ketone body production in perfused liver from the dilution of the specific radioactivities of *R*-[3-^{14}C]BHB and [3-^{14}C]AcAc following addition of a tracer of either *R*-[3-^{14}C]BHB or [3-^{14}C]AcAc to the perfusate. Using

the same concept, we calculated the total rate of ketogenesis, K, from the dilution of the MPE of *R*-[$^{13}C_2$]BHB and [$^{13}C_2$]AcAc after addition of 80 μmol of [3,4-$^{13}C_2$]AcAc to the perfusate. The pool of *R*-BHB + AcAc is considered as a single species whose total MPE, TMPE, is:

$$\text{TMPE} = \frac{a_{R\text{-}[^{13}C_2]\text{BHB}} + a_{[^{13}C_2]\text{AcAc}}}{a_{R\text{-BHB}} + a_{\text{AcAc}} + a_{R\text{-}[^{13}C_2]\text{BHB}} + a_{[^{13}C_2]\text{AcAc}}} \quad (5)$$

The rate of ketogenesis, K (μmol/min), is calculated from the linear plot of 1/TMPE versus time (t):

$$1/\text{TMPE} = (1 + Kt/M_0)/\text{TMPE}_0 \quad (6)$$

where M_0 is the pool size (μmol) of *R*-BHB + AcAc, and TMPE_0 is the total MPE of this pool, both extrapolated to just after addition of the tracer. Eqn. (6) is similar to that developed for measuring ketogenesis with radioactive tracers [18]. In some experiments, 1/TMPE was not linear throughout the experiment. In this case ketogenesis was calculated by summation of rates calculated during the intervals between sampling times.

Cumulative ketone body accumulation, expressed in μmol/90 min per g dry weight, was calculated as:

$$\text{Ketone body accumulation} = (\mu\text{mol of } R\text{-BHB} + \text{AcAc})_{t-120\text{ min}} - (\mu\text{mol of } R\text{-BHB} + \text{AcAc})_{t-30\text{min}} \quad (7)$$

The absolute contribution of 1,3-butanediol metabolism to total ketogenesis, expressed as a percentage, was calculated from the ^{14}C labelling of perfusate *R*-BHB and AcAc as:

$$\text{Contribution (\%)} = \frac{\text{Final SA of [}^{14}\text{C]AcAc} \times (\mu\text{mol of } R\text{-BHB} + \text{AcAc in perfusate}) \times 100}{\text{SA of [}^{14}\text{C]1,3-butanediol}} \quad (8)$$

where SA is specific radioactivity.

Acetone production

The production of acetone was calculated using the first-order kinetic constant of AcAc decarboxylation (K_{dc}) determined previously in perfused rat liver [23]. K_{dc} (expressed in min^{-1}) has two components, one for the perfusate and one for the liver:

$$K_{dc} = 0.0015 + 0.00067 \times \text{liver wet wt. (g)} \quad (9)$$

For each time interval Δt between samples, the production of acetone was calculated by:

$$\text{Production} = K_{dc} \times \overline{[\text{AcAc}]}_{\Delta t} \times \text{perfusate volume} \quad (10)$$

where $\overline{[\text{AcAc}]}_{\Delta t}$ is the calculated AcAc concentration in the middle of the Δt interval. The cumulative acetone production was calculated by summation of production in sequential intervals.

The incorporation of *R*- or *S*-[3-^{14}C]1,3-butanediol into acetone during each time interval, Δt, was calculated as:

$$\text{Incorporation} = \frac{(\text{Total acetone production})_{\Delta t} \times (\text{average SA of C-3 of AcAc})_{\Delta t}}{\text{SA of [3-}^{14}\text{C]1,3-butanediol}} \quad (11)$$

During experiments with *S*-[3-^{14}C]1,3-butanediol, AcAc became labelled in C-1. Thus a fraction of $^{14}CO_2$ production arose from AcAc decarboxylation. During each Δt, $^{14}CO_2$ production from AcAc decarboxylation was calculated as:

$$(^{14}CO_2 \text{ production from AcAc})_{\Delta t} = (\text{total acetone production})_{\Delta t} \times (\text{average SA of C-1 of AcAc})_{\Delta t} \quad (12)$$

The cumulative percentage contribution of AcAc decarboxylation to $^{14}CO_2$ production was:

$$\text{Contribution (\%)} = \frac{\Sigma(^{14}CO_2 \text{ production from AcAc})_{\Delta t} \times 100}{\text{Total } ^{14}CO_2 \text{ production}} \quad (13)$$

RESULTS AND DISCUSSION

The cumulative *R*- and *S*-1,3-butanediol uptakes (Table 1), measured in livers from fed rats, were 380–386 μmol/90 min g dry wt. These were not significantly different from the cumulative *R*- and *S*-1,3-butanediol uptakes of livers from starved rats, which were 355 and 409 μmol/90 min per g dry wt. respectively. Note that the initial concentration of the 1,3-butanediol isomers was 50% greater in perfusions of livers from starved rats than in perfusions of livers from fed rats. Lincoln has shown [30] that rat liver alcohol dehydrogenase exhibits identical K_m (1.48 mM) and $V_{max.}$ (1 μmol/min per g of liver) values for *R*- and *S*-1,3-butanediol. In the present study, we show (Table 1) that *R*- and *S*-1,3-butanediol are taken up at identical rates by perfused livers from fed and starved rats. Thus differences in the metabolism or metabolic effects of *R*- and *S*-1,3-butanediol must be ascribed to events occurring after their oxidation to *R*- or *S*-BHB.

Ketogenesis was measured by isotope dilution and substrate balance. Fig. 1(*a*) shows the profile of equilibration of the MPEs of *R*-BHB and AcAc, following the addition of [3,4-$^{13}C_2$]AcAc to the perfusate. Total ketogenesis was calculated (eqn. 6) from the dilution of the MPE of the ketone body pool. As previously shown with ^{14}C tracers [18], the inverse of the MPE of the total ketone body pool increases linearly with time (Fig. 1*b*), with a slope proportional to the rate of ketogenesis.

In livers from fed rats, ketogenesis was increased 9- and 3.5-fold by *R*-1,3-butanediol and *S*-1,3-butanediol respectively (Table 2). However, in livers from starved rats, ketone body production was increased 3.5- and 1.5-fold by *R*-1,3-butanediol and *S*-1,3-butanediol, respectively. Accumulation of *R*-BHB + AcAc accounted for 76–96% of ketone body production. We have shown that the difference between total ketogenesis and ketone body accumulation is accounted for by (i) conversion of AcAc to acetone [23], and (ii) incorporation of AcAc into fatty acids, sterols and CO_2 [18].

After addition of *R*-1,3-butanediol to the perfusate, total ketogenesis (measured by isotopic dilution) amounted to 80–102% of *R*-1,3-butanediol uptake (compare rows 1 of Tables 1 and 2). In contrast, in the presence of *S*-1,3-butanediol, total ketogenesis amounted to only 29–38% of *S*-1,3-butanediol uptake. This is consistent with the accumulation of the unnatural enantiomer *S*-BHB. The total production of (i) physiological ketone bodies (*R*-BHB + AcAc), and (ii) *S*-BHB (rows 2 and 3 of Table 1) accounted for 73–78% of *S*-1,3-butanediol uptake (row 1 of Table 1).

When livers were perfused with *R*- or *S*-[3-^{14}C]1,3-butanediol, AcAc became progressively labelled (Fig. 2); labelling of *R*-BHB followed the same pattern (results not shown). In the case of

Table 1. Balance of *R*- and *S*-1,3-[3-^{14}C]butanediol metabolism in rat liver

Livers were perfused with medium containing glucose (fed, 15 mM; starved, 4 mM) in the presence of 5 mM (fed rats) and 7.5 mM (starved rats) of *R*- or *S*-1,3-[3-^{14}C]butanediol. All cumulative rates are expressed as μmol of butanediol/90 min per g dry wt. (means ± S.E.M.). *Differs from corresponding parameter for fed group ($P < 0.05$ using a two-sided t test).

	Fed		Starved	
	R-1,3-Butanediol ($n = 6$)	*S*-1,3-Butanediol ($n = 5$–7)	*R*-1,3-Butanediol ($n = 5$–7)	*S*-1,3-Butanediol ($n = 4$)
Uptake of 1,3-butanediol	380 ± 40	386 ± 22	355 ± 17	409 ± 30
Incorporation of 1,3-butanediol into:				
R-BHB + AcAc	247 ± 31	76.0 ± 7.8	282 ± 9	67.0 ± 7.5
S-BHB	–	224 ± 22	–	231 ± 12
Fatty acids + sterols	5.38 ± 0.57	14.2 ± 1.7	0.21 ± 0.03	0.18 ± 0.01
CO_2	3.35 ± 0.48	11.6 ± 2.4	1.28 ± 0.11	4.07 ± 0.59
Acetone	39.4 ± 6.1	8.70 ± 1.08	27.5 ± 1.5	5.25 ± 0.72*
Total 1,3-butanediol incorporation	294 ± 36	331 ± 31	311 ± 9	307 ± 53
Amount of 1,3-butanediol uptake accounted for (%)	89 ± 13	81 ± 4	89 ± 5	75 ± 4

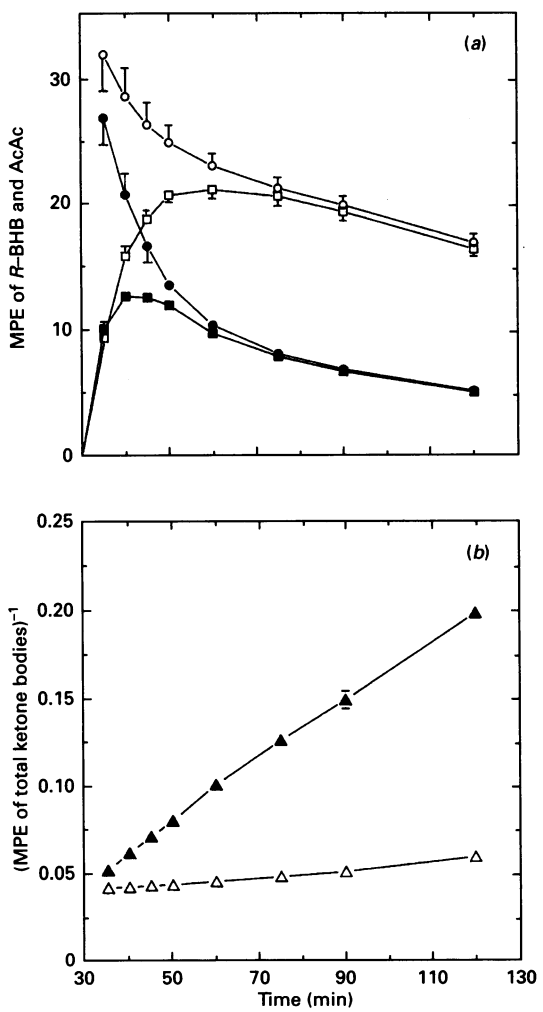


Fig. 1. Profile of the equilibration of the MPE of *R*-BHB (□, ■) and AcAc (○, ●) following addition of a tracer of [3,4-$^{13}C_2$]AcAc to the recirculating perfusate at 30 min

Livers from fed rats were perfused with 15 mM-glucose. Six of the experiments served as controls (○, □). In the other six experiments, 5 mM-*R*-[3-^{14}C]1,3-butanediol was added at 30 min (●, ■). (*a*) MPEs of *R*-BHB and AcAc; (*b*) inverse of the combined MPE of *R*-BHB and AcAc (eqn. 5) for control (△) and *R*-[3-^{14}C]1,3-butanediol (▲) liver perfusions.

livers from fed rats perfused with *R*-[3-^{14}C]1,3-butanediol, the SA of AcAc almost reached a plateau by the end of the experiment. This corresponds to a situation where (i) the pool of unlabelled ketone bodies was small at the time of addition of the diol, and (ii) the rate of production of [^{14}C]ketone bodies from the diol was high. The opposite situation occurred in the case of livers from starved rats perfused with *S*-[3-^{14}C]1,3-butanediol. There, the profile of ketone body labelling remained in the initial linear phase of the saturation curve.

The absolute contribution of 1,3-butanediol to total ketogenesis was calculated from the ^{14}C labelling of perfusate AcAc and *R*-BHB (eqn. 8). *R*-[3-^{14}C]1,3-Butanediol contributed 86% and 98% of total ketogenesis in livers from starved and fed rats respectively (Table 2), and *S*-[3-^{14}C]1,3-butanediol contributed 47% and 75% of total ketogenesis in livers from starved and fed rats respectively. The lower contributions of the diols to ketone body production in livers from starved rats are probably the result of endogenous ketogenesis.

AcAc isolated from the perfusate at each time point was degraded [26] to acetone (C-2 to C-4 of AcAc) and CO_2 (C-1 of AcAc). In livers perfused with *R*-[3-^{14}C]1,3-butanediol, less than 1% of the label was found on C-1 of AcAc. In livers perfused with *S*-[3-^{14}C]1,3-butanediol, however, C-1 of AcAc carried 13.5 ± 0.74% ($n = 7$) and 13.1 ± 0.49% ($n = 4$) of the total AcAc label in livers from fed and starved rats respectively. The fractional ^{14}C labelling of C-1 of AcAc was virtually constant throughout each experiment. Transfer of label from C-3 of *S*-[3-^{14}C]1,3-butanediol to C-1 of AcAc is explained by the following sequence: oxidation of *S*-[3-^{14}C]1,3-butanediol to *S*-[3-^{14}C]BHB → activation to *S*-[3-^{14}C]BHB-CoA → reduction to [3-^{14}C]AcAc-CoA → partial randomization to [1,3-^{14}C]AcAc-CoA via AcAc-CoA thiolase → HMG-CoA → partially randomized [1,3-^{14}C]AcAc.

In livers from fed rats, *R*-1,3-butanediol did not affect fatty acid or sterol synthesis (Table 3), and contributed 13% and 27% of the carbon incorporated into fatty acids and sterols respectively. The preferential incorporation of *R*-1,3-butanediol into sterols over fatty acids demonstrates that lipogenesis from *R*-1,3-butanediol occurs via cytosolic activation of AcAc to AcAc-CoA, an intermediate of sterol synthesis (Scheme 1, reaction 12; [18]). Via cytosolic AcAc-CoA thiolase, label from AcAc-CoA goes to acetyl-CoA and to fatty acids (Scheme 1, reaction 7). The differential labelling of fatty acids and sterols confirms the lack of isotopic equilibrium between cytosolic

Table 2. Ketogenesis from *R*- and *S*-1,3-butanediol in the perfused rat liver

Total ketogenesis (A) was measured by isotope dilution. Ketone body accumulation (B) was measured by the accumulation of *R*-BHB and AcAc in the perfusate. The contribution of 1,3-butanediol to total ketogenesis was calculated from eqn. (8). All cumulative rates are expressed as μmol of butanediol/90 min per g dry wt. (means ± S.E.M.). *Differs from corresponding parameter for *R*-1,3-butanediol ($P < 0.05$ using a two-sided *t* test).

	Control		*R*-1,3-Butanediol		*S*-1,3-Butanediol	
	Fed ($n = 7$)	Starved ($n = 7$)	Fed ($n = 6$)	Starved ($n = 5$)	Fed ($n = 6$)	Starved ($n = 4$)
Total ketogenesis (A)	33.5±4.0	107±9	305±20	361±14	112±14	156±23
Ketone body accumulation (B)	17.9±4.1	61.9±11.6	279±24	347±9	85.4±10.8	126.7±8.6
Ketone body uptake (A−B)	15.6±2.7	45.9±16.4	38.8±5.2	13.7±15.7	31.7±6.3	29.3±20.4
(A−B)/A × 100	53.1±6.1	42.6±4.9	13.8±2.2	2.97±3.82	27.9±5.0	22.3±8.3
Butanediol contribution to total ketogenesis (%)	–	–	97.7±8.3	86.4±3.1	75.2±9.2	47.5±8.7*

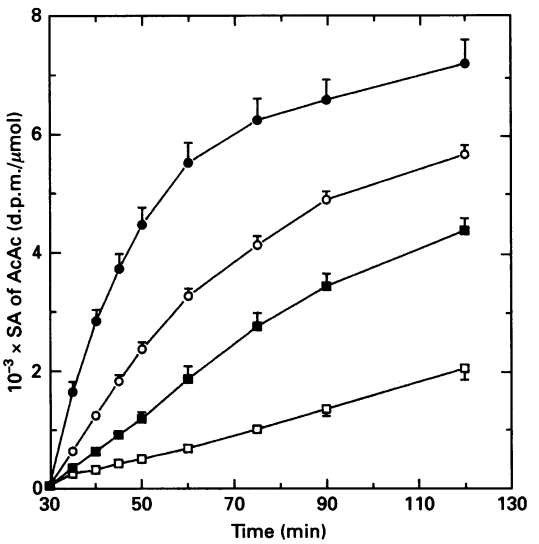


Fig. 2. Labelling of AcAc following addition of 5 mM-*R*-[3-^{14}C]1,3-butanediol (○, ●) and 7.5 mM-*S*-[3-^{14}C]1,3-butanediol (□, ■)

Livers from fed (●, ■) and starved (○, □) rats were perfused with 15 and 4 mM-glucose respectively. At 30 min, [3-^{14}C]1,3-butanediol was added to the perfusate. The SA of *R*-BHB was the same as that of AcAc. Data are presented as means ± S.E.M. ($n = 5$).

AcAc-CoA and acetyl-CoA [18]. In contrast, in livers from fed rats, *S*-1,3-butanediol contributed equally (24%) to both fatty acid and sterol synthesis. This is consistent with incorporation of *S*-BHB via mitochondrial acetyl-CoA → citrate → cytosolic ATP–citrate lyase → cytosolic acetyl-CoA. In this case the cytosolic pool of acetyl-CoA is the only source of label incorporation into fatty acids and sterols [14].

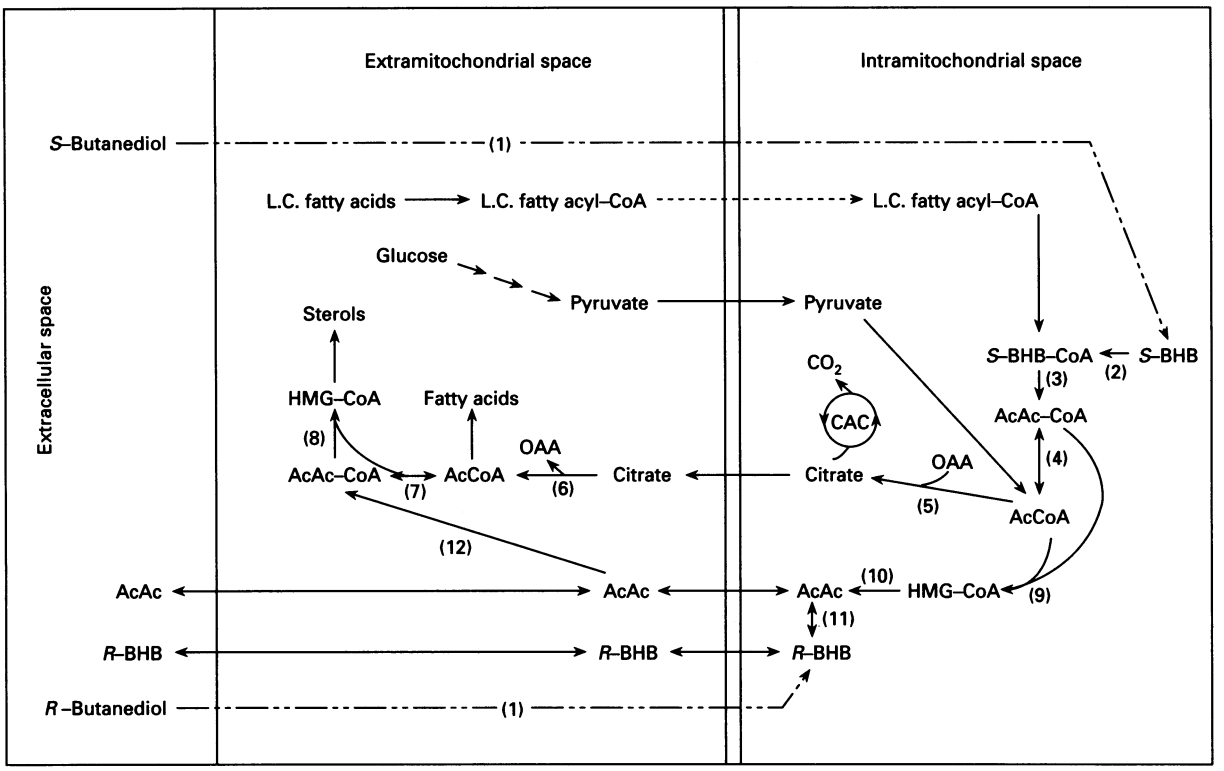
In livers from fed rats perfused with *S*-1,3-butanediol, the increase in fatty acid synthesis over controls was equal to the amount of *S*-1,3-butanediol incorporated (Table 3; rows A and B). Thus it appears that *S*-1,3-butanediol further increases the high fatty acid synthesis occurring in the fed state. In livers from starved rats, fatty acid and sterol synthesis were increased 3.5- and 2.5-fold by *R*- and *S*-1,3-butanediol respectively, but remained well below rates measured in livers from fed rats. Surprisingly, the diols contributed only 6–8% of lipogenic carbon. Thus, in starved rats, both *R*- and *S*-1,3-butanediol must stimulate lipogenesis from other carbon sources, presumably perfusate glucose. In livers from starved rats, the equal contributions of *R*-[3-^{14}C]1,3-butanediol to both total fatty acid and sterol synthesis may reflect isotopic equilibration between cytosolic AcAc-CoA and acetyl-CoA.

Production of $^{14}CO_2$ from *S*-[3-^{14}C]1,3-butanediol was markedly greater than that from *R*-[3-^{14}C]1,3-butanediol (Table 1). In the case of perfusions with *S*-[3-^{14}C]1,3-butanediol, less than

Table 3. Lipogenesis from *R*- and *S*-1,3-butanediol

Livers were perfused with ^3H_2O in the presence of 5 mM (fed rats) or 7.5 mM (starved rats) of *R*- or *S*-1,3-[3-^{14}C]butanediol. All rates are expressed as μmol of acetyl incorporated/90 min per g dry wt. (means ± S.E.M.). Total rates of fatty acid and sterol synthesis (in μmol of acetyl) were obtained by multiplying the μmol of ^{3}H incorporated by 1.15 in the case of fatty acids [28] and by 1.31 in the case of sterols [29]. *Differs from controls ($P < 0.05$ using a two-sided *t* test); †differs from corresponding parameter for *R*-1,3-butanediol ($P < 0.05$ using a two-sided *t* test); ‡differs from corresponding parameter for fatty acid synthesis ($P < 0.05$ using a two-sided *t* test).

	Fed			Starved		
	Control ($n = 6$)	*R*-1,3-Butanediol ($n = 7$–8)	*S*-1,3-Butanediol ($n = 7$–8)	Control ($n = 6$)	*R*-1,3-Butanediol ($n = 7$–8)	*S*-1,3-Butanediol ($n = 5$)
Fatty acid synthesis						
A. Total	85.6±13.4	75.5±12.8	110±16*	1.64±0.16	5.79±0.50*	4.13±0.53*
B. From diol	–	9.00±1.14	25.7±3.20†	–	0.37±0.05	0.32±0.04
C. From other than diol (A−B)	85.6±13.4	66.5±11.2	84.1±13.2	1.64±0.16	5.66±0.48	3.66±0.60*
D. (B/A) × 100	–	12.9±1.0	24.1±1.8†	–	6.26±0.52	7.93±0.30
Sterol synthesis						
E. Total	9.91±1.11	6.97±0.90	10.4±1.5	0.42±0.12	0.81±0.18	0.43±0.19
F. From diol	–	1.76±0.17	2.60±0.46	–	0.06±0.01	0.032±0.011
G. From other than diol (E−F)	9.91±1.11	5.20±0.79*	7.84±1.10	0.42±0.12	0.75±0.16	0.41±0.18
H. (F/E) × 100	–	26.8±2.9‡	24.3±1.9	–	6.78±0.58	7.92±0.78



Scheme 1. Scheme of *R*- and *S*-1,3-butanediol metabolism in the liver

The numbers refer to the following enzymes: 1, alcohol and aldehyde dehydrogenases; 2, *S*-3-hydroxybutyryl-CoA ligase; 3, *S*-3-hydroxybutyryl-CoA dehydrogenase; 4 and 7, mitochondrial and cytosolic AcAc-CoA thiolase respectively; 5, citrate synthase; 6, ATP-citrate lyase; 8 and 9, cytosolic and mitochondrial *S*-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase; 10, HMG-CoA lyase; 11, *R*-3-hydroxybutyrate dehydrogenase; 12, AcAc-CoA synthetase. The scheme does not include spontaneous decarboxylation of AcAc to acetone + CO_2. Abbreviations: CAC, citric acid cycle; L.C., long-chain; OAA, oxaloacetate.

1.4% of the $^{14}CO_2$ production could be ascribed to decarboxylation of partially randomized [1,3-^{14}C]AcAc (see the Materials and methods section). Incorporation of 1,3-butanediol into ketone bodies, *S*-BHB (in the case of *S*-1,3-butanediol), lipids, CO_2 and acetone accounted for 75–89% of uptake of the diols.

Nakagawa *et al.* [10] reported a marked decrease (from 25 to 8 mM) in the glycaemia of alloxan-diabetic rats fed a diet containing 6% of the energy as *S*-1,3-butanediol. This was not observed with a control diet or a diet containing 6% of the energy as *R*-1,3-butanediol. Since *R*- and *S*-1,3-butanediol are oxidized at the same rate by the liver, one cannot ascribe the effect of *S*-1,3-butanediol on the glycaemia of diabetic rats to inhibition of gluconeogenesis by a mechanism similar to that occurring after ingestion of ethanol (i.e. displacement of the equilibrium of some dehydrogenases by an increase in the [NADH]/[NAD$^+$] ratio). Further work is needed to identify the mechanism of the anti-diabetic effect of *S*-1,3-butanediol.

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REFERENCES

1. Miller, S. A. & Dymsza, H. A. (1967) J. Nutr. **91**, 79–88
2. Stahly, T. S., Crocker, D. B. & Cromwell, G. L. (1980) J. Anim. Sci. **51** (suppl. 1), 222 (abstr. no. 286)
3. Davenport, R. F., Griffith, M. & Watts, A. B. (1967) Poultry Sci. **46**, 1250
4. Hess, G. S. & Young, J. W. (1972) J. Dairy Sci. **55**, 1097–1105
5. Münst, G., Ris-Steiger, M. M., Galeazzi, R. L., Von Wartburg, J. P. & Bircher, J. (1981) Biochem. Pharmacol. **30**, 1987–1997
6. Spence, C. A., Boyd, R. D., Wray, C. D. & Whitehead, D. M. (1985) J. Anim. Sci. **60**, 1280–1287
7. Kies, C., Tobin, R. B., Fox, H. M. & Mehlman, M. A. (1973) J. Nutr. **103**, 1155–1163
8. Tobin, R. B., Mehlman, M. A., Kies, C., Fox, H. M. & Soeldner, J. S. (1975) Fed. Proc. Fed. Am. Soc. Exp. Biol. **34**, 2171–2176
9. Majchrowicz, E., Hunt, W. A. & Piantadosi, C. (1976) Science **194**, 1181–1182
10. Nakagawa, H., Tai, A. & Izumi, M. (1979) Jpn. Kokai Tokkyo Koho 79,138,126 (cf. Chem. Abstr. **92**, 140720j)
11. Fisher, G. & Schleiber, H. (1909) Ber. Dtsch. Chem. Ges. **42**, 1219–1228
12. Lehninger, A. L. & Greville, G. D. (1953) Biochim. Biophys. Acta **12**, 188–202
13. Scofield, R. F., Brady, P. S., Schumann, W. C., Kumaran, K., Ohgaku, S., Margolis, J. M. & Landau, B. R. (1982) Arch. Biochem. Biophys. **214**, 268–272
14. Lincoln, B. C., Des Rosiers, C. & Brunengraber, H. (1987) Arch. Biochem. Biophys. **259**, 149–156
15. Des Rosiers, C., Montgomery, J. A., Desrochers, S., Garneau, M., David, F., Mamer, O. A. & Brunengraber, H. (1988) Anal. Biochem. **173**, 96–105
16. Passingham, B. J. & Barton, R. N. (1975) Anal. Biochem. **65**, 418–421
17. McCann, W. P. (1962) Biochem. Prep. **9**, 63–68
18. Endemann, G., Goetz, P. G., Edmond, J. & Brunengraber, H. (1982) J. Biol. Chem. **257**, 3434–3440
19. Reference deleted
20. Desrochers, S., Montgomery, J. A., Des Rosiers, C., Lincoln, B. C. & Brunengraber, H. (1990) Anal. Biochem. **186**, 101–107
21. Reference deleted
22. Kopito, R. R., Weinstock, S. B., Freed, L. E., Murray, D. M. & Brunengraber, H. (1982) J. Lipid Res. **23**, 577–583

23. Gavino, V. C., Somma, J., Philbert, L., David, F., Garneau, M., Belair, J. & Brunengraber, H. (1987) J. Biol. Chem. **262**, 6735–6740
24. Williamson, D. H. & Mellanby, J. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1836–1839, Academic Press, New York
25. Mellanby, J. & Williamson, D. H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1840–1843, Academic Press, New York
26. Mayes, P. A. & Felts, J. M. (1967) Biochem. J. **102**, 230–235
27. Andersen, J. M. & Dietschy, J. M. (1979) J. Lipid Res. **20**, 740–752
28. Brunengraber, H., Boutry, M. & Lowenstein, J. M. (1973) J. Biol. Chem. **248**, 2656–2669
29. Brunengraber, H., Sabine, J. R., Boutry, M. & Lowenstein, J. M. (1972) Arch. Biochem. Biophys. **150**, 392–396
30. Lincoln, E. C. (1984) Ph.D. Thesis, Massachusetts Institute of Technology

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