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Intraruminal propionate supplementation modifies hindlimb energy metabolism without changing the splanchnic release of glucose in growing lambs

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The influence of propionate supplementation on the splanchnic metabolism of energy-yielding nutrients and the supply of glucose to the hindlimb was investigated in growing lambs. Six rumen-cannulated and multicatheterized lambs (32·2 kg), fed frozen rye grass at 690 kJ metabolizable energy intake/d per kg body weight$^{0·75}$, were infused intraruminally with a salt solution (control) or with propionate solutions at 0·55 mol/d (P1) or 0·98 mol/d (P2) according to a replicated Latin square design. In the rumen fluid, supplementation decreased the acetate:propionate molar ratio from 2·36:1 to 1·37:1, without modifying the ruminal concentrations of acetate and NH_4. As a result, the portal appearance of propionate increased by 51 and 72 % with P1 and P2, respectively, and that of L-lactate doubled. Across the liver, net extraction of propionate increased by 47 and 67 % with P1 and P2, respectively. However, the net hepatic production of glucose remained unchanged, probably as the result of a substantial rise in insulin secretion and its hepatic extraction. Overall, the net splanchnic release of acetate, glucose and butyrate was not modified while that of L-lactate increased. Despite this, the net uptake of acetate, glucose, L-lactate and non-esterified fatty acids by the hindlimb increased. Propionate probably enhanced the storage of energy-yielding nutrients in the hindlimb, despite their unchanged release by the splanchnic tissues and the unmodified insulinaemia. Regulatory mechanisms are not clear.

Propionate: Splanchnic tissues: Hindlimb: Insulin

In European countries, the irregular sensory traits of beef, in particular tenderness, juiciness and flavour, contribute to the decrease in meat consumption (Geay *et al.* 2001). These traits are partly correlated to muscle energy metabolism, in particular the proportion of glycolytic fibres, and resultant energy storage, as glycogen and fat (Pethick & Dunshea, 1997; Hocquette *et al.* 1998). Since glucose is the preferred substrate of glycolytic fibres and the major precursor for glycogen and intramuscular triacylglycerol synthesis (Pethick & Dunshea, 1997; Hocquette *et al.* 1998), both tenderness and juiciness may be positively influenced by glucose supply and metabolism in muscle.

In ruminants, the manipulation of glucose supply to, and utilisation by, muscle is not straightforward. Glucose is poorly absorbed and originates mostly from hepatic gluconeogenesis which varies with metabolizable energy (ME) intake (MEI), the pattern of ruminal fermentation, the supply of gluconeogenic substrates as well as the hormonal status and the energy requirements of the animal (Brockman, 1993; Ndi Bualonji & Godeau, 1993; Lapierre *et al.* 2000; Drackley *et al.* 2001).

Propionate is the major gluconeogenic precursor, accounting for 32–73 % of hepatic glucose synthesis (Seal & Reynolds, 1993), but increased propionate absorption does not proportionally modify glucose supply to muscle. Indeed, it is metabolised in the rumen epithelium, although the extent of this metabolism is controversial (Veenhuizen *et al.* 1988; Seal & Reynolds, 1993; Rémond *et al.* 1995, Kristensen *et al.* 2000*a*). Additionally, the different responses of hepatic gluconeogenesis have been observed following an increased supply of propionate. In growing steers, propionate supplementation (at levels of

Abbreviations: ME, metabolizable energy; MEI, metabolizable energy intake; NEFA, non-esterified fatty acids; NPA, net portal appearance; NPAP, net portal appearance of propionate; P1, 0·23 M-propionate infusion; P2, 0·41 M-propionate infusion; PDV, portal-drained viscera; TG, triacylglycerols; VFA, volatile fatty acids; $W^{0·75}$, metabolic body weight.

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4·2 and 25 % of MEI) increased whole-body glucose turnover by 13 and 59 % (Veenhuizen *et al.* 1988; Seal & Parker, 1994). These responses were associated with increases in arterial insulin concentrations. Conversely, a 3 d intramesenteric infusion of propionate (at 2·4 % of MEI) in lactating cows slightly reduced net hepatic glucose release but did not modify insulinaemia, despite an elevated insulin secretion (Casse *et al.* 1994). The contradictory effects of propionate on hepatic gluconeogenesis can be associated with differences in insulin metabolism (Brockman, 1993; Ndi Bualonji & Godeau, 1993; Eisemann *et al.* 1994; Donkin *et al.* 1997), energy balance or glucose requirements of animals (Drackley *et al.* 2001). At the peripheral level, it is not known to what extent propionate supplementation can modify the metabolism of glucogenic nutrients either directly via changes in nutrient supply or indirectly via changes in hormonal status (Hocquette *et al.* 1998).

We assumed that in rapidly growing lambs, fed grass, an exogenous propionate supply would increase the splanchnic release of glucose and its utilisation by the hindlimb. The experiment reported here was designed to test this hypothesis, while accounting for the known interactions between the metabolism of glucose and that of other energy-yielding nutrients and insulin.

Materials and methods

Animals, diet and treatments

Six INRA 401, early weaned, 3-month-old, male lambs of 31 (SD 4·0) kg were surgically equipped with a rumen cannula (12 mm internal diameter), chronic blood catheters in the portal, hepatic, and external iliac veins as well as a mesenteric artery. Splanchnic catheters were introduced as described by Ortigues *et al.* (1994). The iliac catheter was introduced in the external iliac vein via the femoral vein, such that the tip lay between the deep femoral vein and the circumflex iliac vein. The drained compartment of this hindlimb preparation comprises 47–61 % as muscle (Harris *et al.* 1992). Lambs were also fitted with ultrasonic blood flow probes (Transonic Systems, Ithaca, NY) in the portal vein (16 A) and the external iliac artery (3 R) of the catheterized leg. Animals were housed in individual stalls with *ad libitum* access to drinking water and salt lick, under continuous lighting. After a minimum 1-week recovery period from surgery, lambs were adapted to the basal diet for 2 weeks. They were offered perennial rye grass (first cut, fertilised at 80 kg N/ha, harvested at the grazing stage, chopped to 50 mm lengths, frozen at −35°C and stored at −15°C) at an estimated intake of 690 kJ MEI/kg metabolic body weight ($W^{0·75}$), i.e. approximately 75 % of *ad libitum* intake, in twelve equal meals daily. Rye grass ME content was estimated at 11·59 MJ/kg DM (Institut National de Recherches Agronomiques, 1978).

Lambs (at an average experimental weight of 32 ± 2·2 kg) received three treatments according to a 3 × 3 Latin square design, with two repetitions. Treatments consisted of 8 d of continuous intraruminal infusion of a salt solution for the control treatment, or one of two propionate solutions, at 0·23 M (P1) and 0·41 M (P2). Lambs were adapted progressively to the infusions during the first 3 d (38, 75 and 88 % of the final level) and were infused at the final level during the last 5 d. At that level, propionate infusion supplied either 69 (P1) or 110 (P2) kJ ME/kg $W^{0·75}$/d, respectively. All solutions were buffered to pH 4·6, with HCl for control and with NaOH (3 M) and KOH (1 M) for P1 and P2, and supplied the same daily amounts of Na and K (4·97 g Na/d and 2·82 g K/d). Solutions were infused at a maximal rate of 1·68 ± 0·057 ml/min. Treatment periods were separated by a transition period of 7 d. Preliminary tests conducted in order to set the propionate infusion level and the composition of the infused solutions (pH, concentrations, osmolality) showed that the chosen levels had no effects on feed intake, nor on rumen parameters (pH, osmolality).

The experiment was conducted in a manner compatible with national legislation on animal care (Certificate of Authorisation to Experiment on Living Animals, no. 004495, Ministry of Agriculture).

Measurements

On the last day of infusion, blood flows in the portal vein and in the external iliac artery were continuously recorded over 4 h (over two feeding cycles, between 11.00 and 15.00 hours). Hepatic arterial blood flow was estimated at 5·3 % of the portal blood flow based on the reported contribution to hepatic blood flow (Barnes *et al.* 1986; Milano *et al.* 2000). To facilitate the interpretation of net iliac fluxes, visual observations were made of the behaviour of the animals during this period and blood sampling was performed only in quietly standing animals. Eight sets of blood samples were taken from the portal, hepatic and external iliac veins and from the mesenteric artery, 30 min apart starting 15 min postprandially. For each sample, blood (2 ml) was taken using airtight syringes containing 50 μl of diluted heparin (500 IU heparin/ml saline). Half of the sample (1 ml) was immediately deproteinized with 2 ml of perchloric acid (0·6 M), centrifuged at −4°C and the supernatant fraction stored at −20°C for further analyses of glucose (Bergmeyer *et al.* 1974), L-lactate (Gutmann & Wahlefeld, 1974) and β-hydroxybutyrate (Williamson *et al.* 1962). The other 1 ml portion was rapidly used for the analysis of total O_2 content (Tucker, 1967). The remainder of the blood was pooled and stored at −80°C for later determination of volatile fatty acids (VFA) (Reynolds *et al.* 1986). Additionally, blood (2·5 ml) was taken using EDTA–K (25 μl) as anticoagulant and aprotinine (1:10, v/v) and used for packed cell volume determination before centrifugation at −4°C. The resultant plasma was frozen at −20°C for further analyses of non-esterified fatty acids (NEFA) (Kit NEFA C Wako; Unipath SA), triacylglycerols (TG) (Kit TG PAP 1000; Bio Mérieux, Marcy l'Etoile, France) and insulin (Kit Insulin-CT; Cis Bio International, Gif sur Yvette, France).

Three 20 ml rumen fluid samples were taken every 30 min over one feeding cycle (between 15.00 and 17.00 hours). The pH was measured immediately and 10 ml of filtered rumen fluid were acidified with 1 ml of metaphosphoric acid (5 %, v/v) and frozen at −20°C for subsequent

analysis of VFA by GLC (Jouany, 1982) and NH_4 (Van Eenaeme *et al.* 1969).

Calculations and statistical analyses

Iliac artery blood flows of lambs in the quietly standing state were calculated according to Isserty & Ortigues (1994). Net nutrient fluxes through the portal-drained viscera (PDV), the liver and the hindlimb were calculated as described by Katz & Bergman (1969) and Milano *et al.* (2000). A positive value indicates net release whereas a negative value indicates net uptake. Fractional extraction was calculated as the ratio of the net flux to the inflow of nutrient to the tissue. Tissue energy expenditure (kJ/h) was calculated using the McLean (1986) equation as 20·44 × net O_2 flux (litres/h). Net tissue energy fluxes were calculated using heats of combustion (MJ/mol) of acetate (0·875), propionate (1·535), butyrate (2·191), glucose (2·813), L-lactate (1·360), β-hydroxybutyrate (2·055), NEFA (11·106) and TG (34·945).

The maximum potential contribution of substrates to hepatic glucose production was calculated assuming all C was converted to glucose-C. The maximum potential contribution (%) of energy-yielding nutrients to oxidation in the hindlimb was calculated as net nutrient uptake:total O_2 consumption expressed on an energy basis.

For the splanchnic tissues, data were analysed as planned, according to a replicated Latin square design with treatment, square, animal (square) and period as main factors, despite the fact that measurements could not all be carried out on the same days. However, because of lack of patency for some catheters, results for the hindlimb were based on four animals only and data were analysed by ANOVA using treatment, animal and period as the main factors. Analyses were performed using the general linear model procedure of Statistica version 5·5 (1984–2000; StatSoft, Tulsa, OK). Means were compared by orthogonal contrasts in order to test responses to the presence of propionate (contrast 1: comparison between control and the propionate treatments (P1 + P2)), and to level of propionate (contrast 2: P1 *v.* P2). Differences were declared significant at $P<0·05$. Linear regression procedures of Statistica version 5·5 (1984–2000; StatSoft, Tulsa, OK) were used to determine the relationships between the quantity of propionate infused and net nutrient fluxes across the different tissues.

Results

Correct positioning of catheters and probes was checked at necropsy. The splanchnic catheters of all six animals remained functional during the experimental period; catheters and the portal blood-flow probes were correctly positioned. Across the hindlimb, catheters were correctly located but remained functional in four animals only.

Intake and ruminal fermentation parameters

The organic matter, crude protein, crude fibre and soluble sugars contents of rye grass were 895 (SD 31·3), 206 (SD 3·7), 212 (SD 5·5) and 109 (SD 8·0) g/kg on a DM basis, respectively. Soluble N represented 25·6 % of total N. The DM content of the rye grass offered was measured daily and averaged 196 (SD 25·8) g/kg. The daily variability was due to the storage conditions and resulted in a slightly higher DM intake on day 7 and 8 of infusion (+10·5 %, $P<0·07$) for the control than the P1 and P2 treatments (Table 1). Propionate infusion *per se* had no effects on grass DM intake; there were no refusals during the experimental periods. Amounts of propionate infused daily averaged 0·55 and 0·98 moles for P1 and P2, respectively (Table 1). Consequently, total estimated ME intake for control, P1 and P2 averaged 10·57, 10·36 and 10·89 MJ/d of which 0, 8·1 and 13·8 %, respectively, originated from propionate infusion. Total estimated ME intake was not modified by propionate infusion.

In rumen fluid, propionate infusion resulted in 36 (P1) and 71 % (P2) increases ($P<0·001$) in propionate concentrations, leading to increases in total VFA concentrations (Table 1). Ruminal fluid acetate and butyrate concentrations remained unchanged while caproate concentrations tended to decrease by 51·3 % ($P<0·07$). Consequently, molar proportions of acetate dropped from 59·2 to 53·4 and 51·3 % ($P<0·001$) while those of propionate rose from 24·8 to 33·0 and 37·5 % ($P<0·001$) for the control, P1 and P2 treatments, respectively. Rumen fluid pH and NH_4 concentrations were not modified by propionate infusion and rumen pH was similar to that measured before the infusion (6·44 ± 0·245).

Blood flow and nutrient concentrations

Blood flows in the portal vein and in the external iliac artery were not modified by treatments (Table 2) and averaged 105·5 and 6·9 litres/h, respectively. Packed cell volume was stable during the sampling period and was not modified by treatments, averaging 0·27 (data not shown).

The portal concentration of propionate was higher than hepatic and arterial concentrations. Furthermore, all the concentrations increased with propionate infusion (Table 2) but the increases in portal concentrations were much higher (52 and 63 % for P1 and P2) than those of hepatic (31 and 46 % for P1 and P2, respectively) and arterial concentrations (20 and 30 % for P1 and P2, respectively). Across the hindlimb, iliac vein concentrations of propionate were not modified by treatments. The arterial, portal and hepatic concentrations of acetate and butyrate were not altered (Table 2), whereas iliac vein acetate concentrations decreased by 16 % on average ($P<0·04$). Those of butyrate were not modified. Blood concentrations of glucose and L-lactate were not modified by treatments (Table 2). Propionate infusions affected concentrations of lipid-type metabolites with decreases in β-hydroxybutyrate ($P<0·004$) and NEFA ($P<0·02$) concentrations (by 37 and 11 % on average, respectively) in arterial blood. TG and blood O_2 concentrations remained unaffected by treatments.

Net splanchnic metabolism

Only net portal appearance (NPA) of propionate (NPAP)

Table 1. Dietary intake and rumen fluid parameters measured in lambs fed frozen rye grass with intraruminal infusion of propionate*
(Mean values and standard errors of the mean)

		Treatments					Orthogonal contrasts‡ (*P*)	
	n	Control	P1	P2	SEM†	Treatment effect (*P*)	Propionate	Level
Live weight (kg)	6	32·1	32·5	32·3	0·51	0·59	0·38	0·62
Intake (g/d)								
DM	6	913	823	811	26·9	0·07	0·03	0·78
N	6	31·6	27·7	26·2	1·39	0·05	0·02	0·67
Infused propionate (mol/d)	6	0	0·55	0·98	0·033	0·001	0·001	0·001
Rumen fluid parameters								
pH	6	6·48	6·31	6·36	0·087	0·40	0·21	0·65
NH_3 (mM)	6	9·63	10·56	11·27	0·690	0·29	0·16	0·49
Volatile fatty acids (mM)								
Total volatile fatty acids	6	75·82	77·62	86·24	2·603	0·05	0·09	0·05
Acetate	6	44·69	41·49	44·43	1·748	0·40	0·44	0·27
Propionate	6	18·97	25·80	32·36	1·232	0·001	0·001	0·005
Butyrate	6	8·70	7·10	6·52	0·682	0·12	0·05	0·57
Isobutyrate	6	0·99	0·96	0·84	0·067	0·30	0·33	0·22
Valerate	6	0·91	0·88	0·80	0·052	0·37	0·30	0·33
Isovalerate	6	1·18	1·21	1·11	0·074	0·65	0·85	0·38
Caproate	6	0·37	0·19	0·17	0·056	0·07	0·02	0·84
Volatile fatty acids (molar %)								
Acetate	6	59·20	53·40	51·27	1·099	0·002	0·001	0·20
Propionate	6	24·83	33·00	37·50	1·239	0·001	0·001	0·03
Butyrate	6	11·33	9·31	7·73	0·693	0·02	0·01	0·15
Isobutyrate	6	1·36	1·27	1·01	0·097	0·08	0·1	0·09
Valerate	6	1·18	1·15	0·94	0·081	0·13	0·21	0·09
Isovalerate	6	1·63	1·60	1·34	0·120	0·24	0·33	0·16
Caproate	6	0·48	0·25	0·21	0·060	0·03	0·009	0·62

P1, 0·23 M-propionate infusion; P2, 0·41 M-propionate infusion.
* For details of diets and procedures, see p. 40.
† SEM = $\sqrt{}$(Residual mean square/*n*).
‡ Orthogonal contrasts: Propionate = C *v.* (P1 + P2); Level = P1 *v.* P2.

was affected by treatments and rose by 51 and 72 % with P1 and P2, respectively ($P<0{\cdot}001$, Table 3). NPAP was positively correlated to the quantity of propionate infused (mmol/h) (NPAP = (0·829 × propionate infused) + 47·817; $R^2 = 0{\cdot}69$, $P<0{\cdot}001$). The increment in NPAP with P1 and P2 relative to control corresponded to 1·0 (SD 0·61)- and 0·8 (SD 0·28)-fold the amount of propionate infused, without any significant differences between the two levels of propionate. Net PDV flux of glucose was negative (Table 3) representing a fractional extraction of 2·1, 1·7 and 2·5 (SEM 0·4) % of arterial blood glucose with control, P1 and P2, respectively and was not affected by treatments. In contrast, NPA of L-lactate doubled ($P<0{\cdot}03$) with propionate infusion (Table 3).

NPA of β-hydroxybutyrate decreased by 26 and 50 % with P1 and P2, respectively (Table 3), in relation to the reduction of arterial concentrations since net PDV appearance rates of β-hydroxybutyrate (22 (SEM 6·4) % on average) remained unchanged across treatments. These effects may be associated with tendencies for lower rumen butyrate concentrations. NPA of NEFA decreased ($P<0{\cdot}007$) with propionate infusion (Table 3) and was associated with a slight decrease in fractional PDV appearance rate from 10 % with control to 6 and 9 (SEM 0·029) % with P1 and P2 ($P<0{\cdot}1$). On the other hand a net uptake of TG was noted across the PDV with control (Table 3). This uptake tended to increase ($P<0{\cdot}06$) with propionate infusion, as reflected by the response in fractional extraction that varied from 3 % with control to 5·5 (SEM 0·36) %, on average. O_2 consumption by the PDV was not changed by propionate infusion (Table 3).

Across the liver, net uptake of all the VFA measured was noted (Table 3). The fractional extraction with control averaged 1 % for acetate, 75 % for propionate and 63 % for butyrate (Table 4). Whereas the net uptake of acetate and butyrate remained unchanged across treatments, that of propionate rose by 48 and 67 % with P1 and P2 ($P<0{\cdot}001$) as a result of a higher inflow of propionate (Table 3). Net hepatic uptake of propionate (mmol/h) was linearly correlated to NPAP (net hepatic uptake of propionate = (−0·873 × NPAP) − 2·996; $R^2 = 0{\cdot}98$, $P<0{\cdot}001$). The fractional extraction of propionate remained unchanged ($P<0{\cdot}21$). Besides propionate, other major hepatic glucose precursors include L-lactate and glycerol. No net hepatic uptake of L-lactate was noted, but rather a tendency for a net release of L-lactate was measured with control (Table 3); the latter was not significantly modified by treatments. Net hepatic uptake of glycerol was low and unaffected by treatments. The net hepatic release of glucose remained remarkably stable across the three treatments (30·2 mmol/h, Table 3).

Amongst the other metabolites of interest, NEFA were taken up by the liver at approximately 10 % of inflow, with control. The net hepatic release of β-hydroxybutyrate (6·5 mmol/h) with control was reduced ($P<0{\cdot}04$) by 12 and 74 % with P1 and P2, respectively. O_2 consumption by the liver was not altered by propionate infusion (Table 3).

Overall, the net splanchnic release of acetate

Table 2. Blood flows (litres/h) and nutrient concentrations (mM) in lambs fed frozen rye grass with intraruminal infusion of propionate*
(Mean values and standard errors of the mean)

		Treatments					Orthogonal contrasts‡ (*P*)	
	n	Control	P1	P2	SEM†	Treatment effect (*P*)	Propionate	Level
Blood flow								
Portal vein	6	105·5	101·0	106·0	3·13	0·65	0·69	0·41
External iliac artery	4	6·9	6·9	6·8	0·24	0·80	0·56	0·83
Arterial blood								
Blood								
O_2	6	6·50	6·65	6·60	0·112	0·79	0·54	0·82
Acetate	6	1·57	1·52	1·46	0·053	0·56	0·36	0·60
Propionate	6	0·10	0·12	0·13	0·004	0·003	0·002	0·04
Butyrate	6	0·02	0·02	0·01	0·001	0·45	0·93	0·22
Glucose	6	3·10	3·05	3·07	0·060	0·82	0·57	0·81
L-lactate	6	0·70	0·71	0·74	0·039	0·83	0·68	0·66
β-Hydroxybutyrate	6	0·41	0·27	0·25	0·085	0·01	0·004	0·72
Plasma								
Non-esterified fatty acids	6	0·09	0·08	0·08	0·014	0·05	0·02	0·41
Triacylglycerols	6	0·02	0·02	0·01	0·001	0·15	0·08	0·36
Portal blood								
Blood								
O_2	6	4·66	4·88	4·90	0·117	0·28	0·12	0·88
Acetate	6	2·64	2·44	2·50	0·080	0·54	0·31	0·72
Propionate	6	0·54	0·82	0·88	0·040	0·001	0·001	0·14
Butyrate	6	0·08	0·08	0·07	0·004	0·82	0·82	0·57
Glucose	6	3·03	3·00	2·99	0·060	0·85	0·58	0·94
L-lactate	6	0·74	0·80	0·82	0·046	0·62	0·36	0·78
β-Hydroxybutyrate	6	0·50	0·34	0·29	0·096	0·007	0·002	0·42
Plasma								
Non-esterified fatty acids	6	0·10	0·09	0·08	0·014	0·03	0·009	0·65
Triacylglycerols	6	0·02	0·02	0·01	0·004	0·14	0·06	0·53
Hepatic blood								
Blood								
O_2	6	3·45	3·47	3·47	0·094	0·99	0·92	0·97
Acetate	6	2·55	2·37	2·41	0·082	0·52	0·28	0·81
Propionate	6	0·13	0·17	0·19	0·009	0·001	0·000	0·10
Butyrate	6	0·03	0·03	0·03	0·002	0·88	0·81	0·67
Glucose	6	3·31	3·28	3·29	0·060	0·84	0·58	0·90
L-lactate	6	0·74	0·81	0·84	0·044	0·57	0·32	0·78
β-Hydroxybutyrate	6	0·55	0·38	0·31	0·105	0·005	0·002	0·19
Plasma								
Non-esterified fatty acids	6	0·09	0·08	0·08	0·010	0·03	0·009	0·79
Triacylglycerols	6	0·02	0·01	0·02	0·004	0·10	0·04	0·58
Iliac venous blood								
Blood								
O_2	4	3·08	2·75	2·76	0·158	0·56	0·31	0·97
Acetate	4	0·82	0·64	0·73	0·062	0·06	0·04	0·14
Propionate	4	0·10	0·08	0·09	0·001	0·50	0·30	0·63
Butyrate	4	0·01	0·01	0·01	0·009	0·74	0·74	0·51
Glucose	4	2·88	2·82	2·68	0·061	0·34	0·31	0·28
L-lactate	4	0·70	0·69	0·65	0·059	0·97	0·83	0·97
β-Hydroxybutyrate	4	0·28	0·22	0·16	0·129	0·04	0·03	0·10
Plasma								
Non-esterified fatty acids	4	0·10	0·08	0·07	0·011	0·05	0·02	0·44
Triacylglycerols	4	0·02	0·02	0·01	0·003	0·14	0·06	0·80

P1, 0·23 M-propionate infusion; P2, 0·41 M-propionate infusion.
* For details of diets and procedures, see p. 40.
† SEM = $\sqrt{}$(Residual mean square/*n*).
‡ Orthogonal contrasts: Propionate = C *v.* (P1 + P2); Level = P1 *v.* P2.

(102·6 mmol/h) and butyrate (1·3 mmol/h) remained unmodified by treatments, while propionate increased ($P<0·02$) (Table 3). Net glucose release to peripheral tissues remained constant across all three treatments (23·8 mmol/h, Table 3). In contrast, net splanchnic L-lactate doubled with propionate infusion ($P<0·02$). The net splanchnic release of β-hydroxybutyrate with control (15·6 mmol/h) decreased ($P<0·004$) with propionate infusions by 20·0 and 60·1 % with P1 and P2, respectively. On the other hand, small net splanchnic uptakes of NEFA and TG were measured with control (0·13 and 0·07 mmol/h, respectively), and despite a 2·8- and 2·4-fold increase

Table 3. Nutrient net fluxes (mmol/h) across the portal-drained viscera (PDV), liver and splanchnic bed in lambs fed frozen rye grass with intraruminal infusion of propionate*

(Mean values and standard errors of the mean)

		Treatments					Orthogonal contrasts‡ (*P*)	
	n	Control	P1	P2	SEM†	Treatment effect (*P*)	Propionate	Level
PDV net fluxes								
Blood								
O_2	6	−197·39	−179·61	−178·88	7·814	0·34	0·15	0·96
Acetate	6	112·93	93·19	109·20	5·488	0·37	0·36	0·28
Propionate	6	46·35	70·09	79·68	3·983	0·003	0·001	0·19
Butyrate	6	6·40	6·11	6·12	0·387	0·95	0·76	0·99
Glucose	6	−6·74	−5·68	−8·18	1·222	0·78	0·95	0·49
L-lactate	6	4·07	9·08	8·57	1·056	0·02	0·03	0·82
β-Hydroxybutyrate	6	9·12	6·76	4·54	0·737	0·008	0·005	0·07
Plasma								
Non-esterified fatty acid	6	0·64	0·31	0·48	0·070	0·009	0·007	0·06
Triacylglycerols	6	−0·04	−0·10	−0·05	0·014	0·03	0·06	0·03
Hepatic net fluxes								
Blood								
O_2	6	−143·98	−158·00	−167·79	6·657	0·52	0·31	0·64
Acetate	6	−3·25	−2·88	−4·36	3·175	0·96	0·94	0·80
Propionate	6	−43·50	−64·21	−72·48	3·521	0·002	0·001	0·18
Butyrate	6	−5·04	−4·97	−4·80	0·311	0·96	0·84	0·84
Glucose	6	30·23	30·23	31·58	1·673	0·93	0·85	0·74
L-lactate	6	1·08	2·04	1·61	0·840	0·83	0·6	0·79
β-Hydroxybutyrate	6	6·51	5·75	1·69	0·777	0·01	0·04	0·01
Plasma								
Non-esterified fatty acids	6	−0·77	−0·68	−0·61	0·051	0·13	0·08	0·32
Triacylglycerols	6	−0·03	−0·07	−0·04	0·012	0·29	0·22	0·32
Splanchnic net fluxes								
Blood								
O_2	6	−341·36	−337·61	−346·64	11·526	0·96	0·98	0·78
Acetate	6	109·69	90·31	104·84	6·182	0·32	0·29	0·27
Propionate	6	2·85	5·88	7·19	0·751	0·05	0·02	0·39
Butyrate	6	1·36	1·13	1·33	0·138	0·61	0·54	0·45
Glucose	6	23·49	24·55	23·40	1·659	0·93	0·87	0·74
L-lactate	6	5·15	11·12	10·18	1·220	0·07	0·02	0·69
β-Hydroxybutyrate	6	15·63	12·51	6·23	1·297	0·003	0·004	0·009
Plasma								
Non-esterified fatty acids	6	−0·13	−0·37	−0·12	0·054	0·02	0·12	0·01
Triacylglycerols	6	−0·07	−0·17	−0·10	0·016	0·008	0·02	0·01

P1, 0·23 M-propionate infusion; P2, 0·41 M-propionate infusion.
* For details of diets and procedures, see p. 40.
† SEM = $\sqrt{}$(Residual mean square/*n*).
‡ Orthogonal contrasts: Propionate = C *v.* (P1 + P2); Level = P1 *v.* P2.

with P1, the actual amounts taken up remained low (Table 3). O_2 consumption by splanchnic tissues remained unaltered (Table 3).

Net splanchnic fluxes of insulin

Arterial plasma concentrations of insulin (0·2 nmol/ml, Table 5) were not modified by propionate infusion. In contrast, net insulin secretion by the PDV (2·02 nmol/h with control) increased by 246 and 119 % with P1 and P2, respectively ($P<0·001$, Table 5). This elevated portal secretion was accompanied by an increase in the net hepatic uptake of insulin ($P<0·001$), with differences between the two levels of propionate ($P<0·004$). Liver fractional extraction of insulin increased from 3 % with control to 16 and 29 % with P1 and P2 ($P<0·001$). Consequently, net splanchnic fluxes of insulin were not consistently modified by propionate infusion. Furthermore, because of high animal variability, net splanchnic fluxes of insulin were not statistically different from zero across the three treatments.

Hindlimb metabolism

Acetate was quantitatively the major energy-yielding nutrient extracted across the hindlimb (Table 6) and this uptake tended to increase by 34 and 11 % with P1 and P2, respectively ($P<0·08$) as a result of higher fractional extraction ($P<0·05$, Table 6). The other VFA also showed net uptakes, but were not altered by treatment (Table 6). Despite the fact that its splanchnic release was not modified, net glucose uptake by the hindlimb (average 1·4 mmol/h with control) increased ($P<0·03$) by 16 and 32 % with P1 and P2, respectively. The fractional extraction of glucose increased ($P<0·02$) from 6 % with control to 8 and 10 % with P1 and P2, respectively. For control,

Table 4. Fractional extraction (%) of nutrients across the liver in lambs fed frozen rye grass with intraruminal infusion of propionate*
(Mean values and standard errors of the mean)

		Treatments					Orthogonal contrasts‡ (*P*)	
	n	Control	P1	P2	SEM†	Treatment effect (*P*)	Propionate	Level
Blood								
O_2	6	27·5	29·84	30·50	0·010	0·58	0·32	0·82
Acetate	6	1·13	0·64	1·75	0·013	0·91	0·98	0·68
Propionate	6	75·16	77·65	77·09	0·007	0·21	0·09	0·69
Butyrate	6	62·63	62·53	61·30	0·013	0·54	0·54	0·37
Glucose	6	−9·20	−9·30	−9·90	0·006	0·91	0·76	0·76
L-lactate	6	−1·70	−2·60	−2·40	0·029	0·83	0·62	0·92
β-Hydroxybutyrate	6	−25·49	−15·33	−5·80	0·053	0·02	0·41	0·007
Plasma								
Non-esterified fatty acids	6	9·98	10·68	9·00	0·021	0·32	0·08	0·32
Triacylglycerols	6	1·72	6·20	4·00	0·055	0·32	0·19	0·43

P1, 0·23 M-propionate infusion; P2, 0·41 M-propionate infusion.
* For details of diets and procedures, see p. 40.
† SEM = √(Residual mean square/*n*).
‡ Orthogonal contrasts: Propionate = C *v.* (P1 + P2); Level = P1 *v.* P2.

glucose uptake was accompanied by a net release of L-lactate with a maximum of 9 % of the glucose extracted by the hindlimb, recycled to L-lactate. However, net L-lactate release switched to net uptake during propionate infusion ($P<0·007$, Table 6) and represented on average 8 % of the inflow of L-lactate.

Finally, as far as the lipid-type metabolites are concerned, net hindlimb uptake of β-hydroxybutyrate was low and was not modified by treatments. Low net hindlimb releases of NEFA and TG were noted with control. These releases decreased ($P<0·002$) with propionate infusion, switching to net uptakes for NEFA (Table 6). O_2 consumption by the hindlimb was not modified by propionate infusion (average 25·0 mmol/h, Table 6). For the hindlimb, insulin net fluxes could not be obtained because the analytical method used was not precise enough to detect small arterio-venous differences.

Discussion

Intraruminal propionate infusion and ruminal parameters

Frozen rye grass was used in order to have a sufficient stock of homogeneous forage over the 2 months duration of the experiment. Present results indicate that the rumen fermentation pattern of the frozen rye grass, including rumen pH and NH_4 concentrations, was similar to that obtained using fresh rye grass (J. Aufrère, unpublished results). However, the proportion of propionate was higher than previously reported for fresh grass (Journet *et al.* 1995; De Visser *et al.* 1997). Possible effects of freezing on grass degradability (Hristov, 1998) or the high feeding frequency (Sutton *et al.* 1986) can not be excluded.

Intraruminal propionate infusion was aimed at modifying the VFA profile in the rumen in order to approach

Table 5. Insulin plasma arterial concentrations (nmol/ml), net fluxes (nmol/h) and fractional extraction (%) through the portal-drained viscera (PDV), the liver and splanchnic tissues in lambs fed frozen rye grass with intraruminal infusion of propionate*
(Mean values and standard errors of the mean)

		Treatments					Orthogonal contrasts‡ (*P*)	
	n	Control	P1	P2	SEM†	Treatment effect (*P*)	Propionate	Level
Arterial concentrations	6	0·2	0·2	0·2	0·01	0·94	0·97	0·73
Net fluxes								
PDV	6	2·0	7·0	4·4	2·89	0·001	0·001	0·02
Liver	6	−0·7	−3·4	−6·2	2·89	0·001	0·000	0·004
Splanchnic tissues	6	1·3	3·6	−1·8	3·55	0·011	0·69	0·004
Extraction rate								
PDV	6	13·2	54·7	27·0	0·14	0·001	0·001	0·008
Liver	6	−3·3	−16·0	−29·5	0·08	0·001	0·000	0·008
Splanchnic tissues	6	8·7	27·8	−9·2	0·15	0·01	0·93	0·001

P1, 0·23 M-propionate infusion; P2, 0·41 M-propionate infusion.
* For details of diets and procedures, see p. 40.
† SEM = √(Residual mean square/*n*).
‡ Orthogonal contrasts: Propionate = C *v.* (P1 + P2); Level = P1 *v.* P2.

Table 6. Nutrient net fluxes (mmol/h) and fractional extraction (%) across the hindlimb in lambs fed frozen rye grass with intraruminal infusion of propionate*

(Mean values and standard errors of the mean)

		Treatments					Orthogonal contrasts‡ (*P*)	
	n	Control	P1	P2	SEM†	Treatment effect (*P*)	Propionate	Level
Net fluxes								
Blood								
O_2	4	−22·04	−26·84	−26·60	1·399	0·47	0·25	0·92
Acetate	4	−4·33	−5·81	−4·81	0·379	0·07	0·08	0·08
Propionate	4	−0·01	−0·19	−0·30	0·064	0·18	0·08	0·68
Butyrate	4	−0·04	−0·05	−0·02	0·007	0·001	0·09	0·001
Glucose	4	−1·42	−1·65	−1·87	0·127	0·05	0·03	0·09
L-lactate	4	0·25	−0·28	−0·33	0·115	0·02	0·007	0·82
β-Hydroxybutyrate	4	−0·48	−0·28	−0·58	0·086	0·26	0·69	0·13
Plasma								
Non-esterified fatty acids	4	0·02	−0·02	−0·01	0·005	0·005	0·002	0·22
Triacylglycerols	4	0·012	0·000	0·004	0·002	0·004	0·002	0·02
Extraction rate								
Blood								
O_2	4	51·28	58·83	58·73	0·024	0·36	0·18	0·98
Acetate	4	44·13	56·96	50·07	0·041	0·05	0·05	0·07
Propionate	4	1·03	25·43	34·45	0·076	0·22	0·10	0·92
Butyrate	4	32·96	42·74	26·36	0·039	0·001	0·11	0·001
Glucose	4	6·50	7·90	9·50	0·006	0·02	0·02	0·04
L-lactate	4	−5·00	7·93	7·87	0·027	0·01	0·005	0·50
β-Hydroxybutyrate	4	19·30	13·50	35·10	0·088	0·08	0·53	0·03
Plasma								
Non-esterified fatty acids	4	−3·29	4·98	3·39	0·009	0·001	0·001	0·13
Triacylglycerols	4	−12·3	2·10	−5·57	0·035	0·02	0·01	0·03

P1, 0·23 M-propionate infusion; P2, 0·41 M-propionate infusion.
* For details of diets and procedures, see p. 40.
† SEM = √(Residual mean square/*n*).
‡ Orthogonal contrasts: Propionate = C *v.* (P1 + P2); Level = P1 *v.* P2.

that obtained with cereal-rich diets, without disturbing intake, the rumen function and the basal production of VFA. With the basal diet being fed at 1.5 times the maintenance energy requirements, propionate infusion had no negative effects on the appetite of animals. The higher MEI than expected (795 *v.* 690 kJ/d per kg $W^{0·75}$), noted with control on the day of sampling and the day before, was due to the variability in the DM content of frozen grass during conservation. As a result, total MEI showed no significant differences between the three treatments, suggesting that the measured responses could be attributed to the modified balance of nutrients in favour of propionate rather than to changes in total energy supply.

Because molar proportions of propionate in the rumen fluid were higher than expected with control, infusion of propionate, especially for P2, elevated those molar proportions above levels frequently reported for ruminants receiving high-concentrate diets (Journet *et al.* 1995). However, these proportions remained physiological because first, Sharp *et al.* (1982) and Moloney (1998) reported similar proportions in steers or sheep receiving high-grain diets and second, in the present study propionate infusion did not affect rumen pH, acetate or iso-acid concentrations. Rumen fluid butyrate concentration tended to be reduced with propionate infusion, as previously noted by Moloney (1998), suggesting a small drop in the protozoal population in the rumen (Journet *et al.* 1995).

Net tissue fluxes of propionate

Associated with the high molar proportions of propionate in the rumen with the control treatment, arterial and portal concentrations of propionate in lambs were higher than the majority of results observed for growing ruminants (Seal *et al.* 1992; Seal & Parker, 1994; Patil *et al.* 1996). Also, NPAP was approximately twice the values usually reported for animals receiving forage-rich diets at similar or higher levels of MEI (cattle, Seal *et al.* 1992; Reynolds *et al.* 1994; Seal & Parker, 1994; sheep, Patil *et al.* 1996) but lower than values obtained for steers receiving grain-rich diets (Harmon & Avery, 1987; Harmon *et al.* 1989). Thus, energy recovered as VFA represented 85 % of the energy absorbed in the portal vein. VFA proportions (C_2:C_3:C_4, 54:39:8) were comparable to those reported for fresh grass (56:32:12, De Visser *et al.* 1997) and intermediate between those of hay-rich diets (63:25:12, Seal & Parker, 1994) and grain-rich diets (43:46:12, Berthelot *et al.* 2002).

Amounts of propionate infused were almost totally (94 % on average) recovered in the portal vein at both levels of infusion. These average portal recovery rates are higher than previously reported (65–80 %, Gross *et al.* 1990; Seal & Parker, 1994). Biases associated with the analytic procedure of VFA are unlikely since analytical recovery rates of acetate, propionate and butyrate in blood samples (*n* 6) following standard VFA overloading

averaged 1·16 (SD 0·106), 1·05 (SD 0·037) and 1·02 (SD 0·062), respectively. Recently Kristensen *et al* (2000*a*,*b*) showed in sheep that the extent of propionate metabolism in the rumen epithelium was low. Here, basal NPAP was already high and probably little supplementary propionate was further metabolised in gut tissues. In the present study, NPA of L-lactate doubled with propionate infusion. Assuming it originated from propionate metabolism, it would represent a maximum of 16 % of the quantity of propionate infused, on average, which is not compatible with the elevated portal recovery of infused propionate. L-lactate is essentially produced from glucose (Gross *et al.* 1990) even during intraruminal propionate infusions (Seal & Parker, 1994). In the present study, the PDV net uptake of glucose was not altered, implying that an increased conversion of glucose into lactate would decrease glucose oxidation, as shown, *in vitro*, by Harmon (1986). Finally, an increased L-lactate production in the rumen cannot be excluded with propionate treatments as noted by Moloney (1998).

Net hepatic propionate uptake was correlated to the quantity of propionate infused in the rumen ($R^2 = 0{\cdot}55$; $P < 0{\cdot}001$) and to the portal recovery of propionate. Although 77 % of total propionate inflow to the liver was extracted, this represented 92 % of the NPA. This confirms that periportal hepatocytes *in vivo* are not metabolically saturated at portal propionate concentrations below 1 mM (Armentano, 1992). In addition, in the present study, the arterial concentrations of vitamin B_{12} were not limiting and dropped with propionate infusion (Girard *et al.* 2001), which suggest that propionate extracted by the liver was metabolised. Splanchnic release of propionate increased linearly with propionate infusion ($R^2 = 0{\cdot}55$; $P < 0{\cdot}001$) representing on average 8 % of NPAP. An increasing proportion of it was extracted by the carcass, with propionate supplementation, probably for the synthesis and deposition of odd-numbered fatty acids (Berthelot *et al.* 2001). These values confirm data reported by Bergman (1990), that quantitatively the metabolism of the supplementary propionate takes place essentially in the liver (80 %) and to a limited extent in the PDV (6 %) and in the carcass (13 %).

Propionate supply, gluconeogenesis and insulin secretion

The most critical effect of propionate infusion was the increase in insulin secretion and in its hepatic extraction. With control, arterial concentrations of insulin and its secretion were similar to data reported for ruminants receiving a mixed diet (0·17–0·23 nmol/ml; 0·06–0·07 nmol/h per kg body weight, respectively, Eisemann & Huntington, 1994; Seal & Parker, 1994). An increase in insulin secretion after an increased supply of propionate has been reported (Harmon, 1992; Casse *et al.* 1994; Seal & Parker, 1994), but the portal secretion obtained was lower than present results, probably because of a lower supply in propionate. Hepatic extraction of insulin represented 33 % of PDV insulin secretion for the control. This rose to 49 % and 140 % with P1 and P2. Eisemann *et al.* (1994) reported a high correlation between insulin secretion and its hepatic extraction in growing euglycaemic steers infused with insulin. However, these effects have never been reported previously in relation to propionate supplementation. Variable pancreatic insulin secretion and hepatic extraction might explain some of the contradictory results reported in the literature on the effects of propionate supplementation on hepatic gluconeogenesis. Indeed, Eisemann & Huntington (1994) observed significant decreases in hepatic glucose production with intramesenteric insulin infusions, suggesting an inhibitory effect of insulin on gluconeogenesis. The effect of propionate on hepatic glucose production may be influenced by the glucose requirements of animals. This aspect was widely described in lactating cows (Drackley *et al.* 2001). In growing animals, Veenhuizen *et al.* (1988) reported similar regulation when glucose requirements increased experimentally by injecting phlorizin.

In the present study, hepatic production of glucose was not modified despite increases in hepatic propionate uptake, unchanged fractional extraction of and increases in L-lactate and alanine availability (I Savary-Auzeloux, L Majdoub, N Le Floc'h, M Vermorel and I Ortigues-Marty, unpublished results). All the glucose synthesised in the liver could potentially originate from propionate. However, contributions from other precursors such as L-lactate, glycerol or amino acids (I Savary-Auzeloux, L Majdoub, N Le Floc'h, M Vermorel and I Ortigues-Marty, unpublished results) were not modified contrary to previously published results (Baird *et al.* 1980; Brockman, 1985; Casse *et al.* 1994). Instead, one may assume that the metabolic fate of propionate must have been altered as also suggested by Eisemann & Huntington (1994), after intravenous infusion of insulin in euglycaemic growing steers. Theoretically, propionate extracted by the liver can be converted to L-lactate (Brockman, 1993), but only to a limited extent (Demigné *et al.* 1991). Indeed, present results suggest that even with P1 and P2, a maximum of 5 % of propionate could be converted in L-lactate in the liver. Propionate can also be used for hepatic glycogen synthesis (Pethick, 1993), either directly or after conversion into L-lactate or glucose (Eisemann *et al.* 1994) and this metabolism is enhanced *in vitro* with propionate supply, via insulin (Morand *et al.* (1990). Some enhancement of propionate oxidation could also be assumed (Eisemann & Huntington, 1994), but not to a sufficient extent as to modify O_2 consumption. Finally, hepatic synthesis and release of methylmalonate from propionate cannot be overlooked. However the importance of this metabolic pathway appears to be limited (Berthelot *et al.* 2002). Consequently the possible modifications in the metabolic fate of propionate and other glucose precursors in the liver remain unclear.

Propionate infusion also probably stimulated lipid deposition in mesenteric tissues, because NPA of NEFA decreased while net uptake of TG tended to increase. This effect could also be insulin-mediated because Seal & Parker (1994) reported the existence of insulin receptors in the mesenteric adipose tissue.

Modifications in hindlimb net energy fluxes by propionate supplementation

Despite the unchanged splanchnic release of energy as

glucose, L-lactate, VFA, β-hydroxybutyrate, TG and NEFA (202 kJ/h), energy uptake by the hindlimb increased from 8 to 11 and 12 kJ/h (corresponding to 2, 3 and 3 % of MEI) for control, P1 and P2, respectively. This increase was associated mainly with increases in fractional extraction and net uptakes of glucose, acetate and L-lactate. In particular, glucose uptake by the hindlimb increased in a dose-dependent manner with propionate supplementation. With control, 79 % of net glucose splanchnic release was extracted by carcass musculature, rising to 88 and 105 % with P1 and P2, respectively. Net uptake of acetate by carcass musculature followed a similar pattern.

The anomaly of higher hindlimb extraction from the same splanchnic release may be explained in several ways. First, there might have been an increased release of nutrients by other organs or tissues. Kidneys are known to synthesise 5 to 15 % of glucose turnover (Kaufman & Bergman, 1971; Brockman, 1993), in particular from L-lactate and propionate (Kaufman & Bergman, 1974; Watford *et al.* 1987). An increase in renal glucose synthesis would be coherent with the measured increase in the net splanchnic release of L-lactate and propionate. Indeed if all the increment in the net splanchnic L-lactate and propionate release not taken up by carcass tissues were metabolised by kidneys, this would allow a renal synthesis of 5 mmol glucose/h with propionate treatments. This amount is of a similar order of magnitude as the increment of glucose extracted by the total muscle mass with propionate supplementation.

Second, a redistribution of splanchnic-released energy-yielding nutrients between the hindlimb and other peripheral tissues (which are not included in the tissue compartment isolated by the surgical preparation) may occur. This might involve either direct or indirect actions of insulin. Insulin enhances glucose uptake, oxidation and its incorporation into glycogen across the hindlimb (Brockman & Larveld, 1986; Hocquette *et al.* 1998). The uptake of glucose, L-lactate, propionate and, to a lower extent, acetate by the hindquarters was also increased after an intravenous infusion of insulin in growing steers (Prior *et al.* 1984; Eisemann & Huntington, 1994). However, these studies all reported increased arterial concentrations of insulin, which was not the case in the present study. Could the responsiveness of muscle or subcutaneous adipose tissue to insulin have been modified? Approximately half of the propionate-induced insulin response in sheep is mediated via the parasympathetic nervous system (Sano *et al.* 1993), which seems in laboratory animals to modulate muscle responsiveness to insulin (Xie & Lautt, 1996). Tissue sensitivity to insulin was greater for high-concentrate diets than for high-forage diets (Sano *et al.* 1992), but it was reduced by supplementary propionate (Sano & Teashima, 1998), which does not fit with our hypothesis. Differences in the patterns of absorption of propionate during the day could alter the responses to propionate, whether absorbed from the basal diet or as supplement (Moloney, 1998).

In the present study, the higher uptake of energy-yielding nutrients by the hindlimb without any rise in energy expenditure suggests an enhancement in the storage of nutrients as fat or glycogen. This hypothesis would be supported by the increase in NEFA uptake and the decrease in glycerol release (Brockman & Larveld, 1986; Scollan & Jessop, 1995). Acetate and L-lactate are carbon precursors for lipogenesis in extra- and intramuscular adipocytes, and lipogenesis from acetate is enhanced by the presence of glucogenic substrates such as glucose and L-lactate (Prior & Scott, 1980; Smith & Crouse, 1984; Scollan & Jessop, 1995) which provide reducing equivalents and glycerol (Hocquette *et al.* 1998). Assuming that all the increment in net acetate uptake by the carcass noted with P2 relative to control (152 mmol/d) was incorporated into triacylglycerols, approximately 5·5 g TG/d could be synthesised. This would require 91 mmoles glucose/d for NADPH and glycerol synthesis, which would correspond to 64 % of the increment in glucose uptake. The remaining glucose taken up could be incorporated into glycogen (potentially 8·4 g glycosyl units/d) or be used for fatty acid synthesis in intramuscular adipocytes (Smith & Crouze, 1984; Pethick & Dunshea, 1997).

Conclusions

In conclusion, intraruminal propionate infusion proportionally increased NPAP. The main effect of propionate was to stimulate pancreatic secretion and the net hepatic extraction of insulin. In the present study, it is difficult to dissociate the effect of propionate on net nutrient fluxes in the PDV, the liver and the hindlimb from those of insulin, but it is interesting to note that in growing ruminants propionate seems to influence insulin metabolism in the same way as glucose does in single-stomached animals. In the present study, the lack of enhanced hepatic gluconeogenesis in response to higher propionate supply differed from numerous published results. The present study also offers an apparent contradiction between an unchanged splanchnic release of energy-yielding nutrients and an enhanced energy uptake by hindlimb in favour of increased energy depots. The reasons for such a discrepancy are not clear and mechanisms by which peripheral utilisation of nutrients could be modified despite a stable supply would be worth exploring. Consequences of propionate supplementation on meat quality from grass-fed animals may be inferred with probably an orientation of the profile of energy-yielding nutrients across the hindlimb to a glycogenic type, associated in particular to changes in the metabolism of minor substrates (lactate, β-hydroxybutyrate, butyrate). Direct experimental evidence on these quality traits should be obtained.

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