Effects of lactation on L-leucine metabolism in the rat

Studies *in vivo* and *in vitro*

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1. The turnover rate of L-[1-¹⁴C]leucine was increased by 35% in lactating rats compared with virgin rats. Starvation or removal of pups (24 h) returned the value to that of the virgin rat. 2. Incorporation of L-[U-¹⁴C]leucine into lipid and protein of mammary glands of lactating rats *in vivo* increased 7-fold and 6-fold respectively compared with glands of virgin rats. Lactation caused no change in the incorporation of L-[U-¹⁴C]leucine into hepatic lipid and protein. 3. The production of ¹⁴CO₂ from L-[1-¹⁴C]leucine (in the presence of glucose) was similar in isolated acini from glands of fed (chow) and starved lactating rats. Feeding with a 'cafeteria' diet caused a slight decrease, and removal of pups a large decrease, in the oxidative decarboxylation of leucine. 4. Oxidation of L-[2-¹⁴C]leucine to ¹⁴CO₂ was increased about 3-fold in acini from starved lactating rats or lactating rats fed on a 'cafeteria' diet compared with rats fed on a chow diet. Insulin decreased the formation of ¹⁴CO₂ in all three situations. 5. Incorporation of L-[U-¹⁴C]- and [2-¹⁴C]-leucine into lipid was decreased in acini from starved lactating rats and lactating rats fed on a 'cafeteria' diet. Insulin tended to increase the conversion of [2-¹⁴C]leucine into lipid, but this was significant only in the case of the acini from 'cafeteria'-fed rats. 6. Experiments with (–)-hydroxycitrate indicate that the major route for conversion of leucine carbon into lipid in acini is via citrate translocation from the mitochondria. 7. The physiological implications of these findings are discussed.

It is now well established that L-leucine can be utilized by a variety of peripheral tissues of the rat, including heart (Buse *et al.*, 1972; Sans *et al.*, 1980; Tischler & Goldberg, 1980), diaphragm (Manchester, 1965; Odessey & Goldberg, 1972; Buse *et al.*, 1973), skeletal muscle (Odessey & Goldberg, 1972; Adibi, 1976; Hutson *et al.*, 1980), brain (Chaplin *et al.*, 1976; Palaiologos *et al.*, 1979), kidney (Ichihara *et al.*, 1972; Mitch & Chan, 1979) and adipose tissue (Feller & Feist, 1962; Goodman, 1963, 1964, 1977). The first two steps in leucine catabolism are transamination (branched-chain amino acid aminotransferase, EC 2.6.1.6) followed by oxidative decarboxylation (branched-chain oxo acid dehydrogenase, EC 1.2.4.4). The branched-chain oxo acid dehydrogenase is thought to be the rate-limiting step in leucine catabolism and may be regulated by a phosphorylation–dephosphorylation mechanism in certain tissues (Parker & Randle, 1978*a,b*; Odessey & Goldberg, 1979; Hughes & Halestrap, 1980). The end-products of leucine catabolism are acetoacetate and acetyl-CoA, which

can either be completely oxidized or act as precursors for the synthesis of lipid *de novo* (Feller & Feist, 1962).

Previous studies have shown that the pathway of L-leucine catabolism is active in the lactating mammary gland of the rat (Ichihara *et al.*, 1972) and that the amino acid can act as a substrate for lipid synthesis in slices of this tissue (Abraham *et al.*, 1964). The present paper is concerned with a quantitative assessment of the metabolism of L-leucine and of the factors that may affect it in isolated acini from mammary glands of lactating rats. In addition, the effects of lactation on the turnover of leucine and its conversion into protein and lipid of mammary gland and liver *in vivo* are reported.

Experimental

Materials

Rats. Lactating rats of the Wistar strain (250–325 g) with nine or ten pups were used at peak

lactation (10–14 days); virgin rats weighed 190–210 g. Rats were anaesthetized with Nembutal (50 mg/kg body wt.) and the pups were allowed to continue to suckle to maintain prolactin secretion (Amenomori *et al.*, 1970) before removal of the inguinal mammary glands. The rats were fed *ad libitum* on Oxoid breeding diet (Oxoid, London S.E.1, U.K.). One group of rats was fed with 'cafeteria diet', in which the animals were offered a variety of palatable high-energy foods in addition to their normal chow diet. This type of diet induces hyperphagia, eventual development of obesity (Sclafani & Springer, 1976; Rolls *et al.*, 1980) and impaired lipogenesis in mammary glands of lactating fed rats (Agius *et al.*, 1980). Starved rats (with pups if lactating) were deprived of food for 24 h. Some lactating rats had their pups removed for 24 h and are referred to in the text as 'weaned' rats.

Biochemicals. All enzymes, including collagenase (grade II), and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Purified insulin was a gift from Dr. N. Lazarus, Wellcome Research Laboratories, Beckenham, Kent, U.K., and (–)-hydroxycitrate from Dr. Ann Sullivan, Hoffman–La Roche, Nutley, NJ, U.S.A.

Radioactive compounds. D-[1-¹⁴C]Glucose, L-[1-¹⁴C]leucine and L-[U-¹⁴C]leucine were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. L-[2-¹⁴C]leucine was obtained from C.E.A., Gif-sur-Yvette, France.

Methods

Studies in vivo. Anaesthetic was administered 3–5 min before the start of the experiments. [1-¹⁴C]leucine or [U-¹⁴C]leucine (0.5 ml containing 5 μ Ci, with a specific radioactivity of 50 mCi/mmol or 300 mCi/mmol respectively) was injected into the tail vein. Rats were separated from their litters and kept warm on a heated stage maintained at 40°C. Blood (0.2 ml) was collected from the tip of the tail into heparinized tubes at 1, 2, 3, 4, 5, 6, 7 and 20 min after injection. Blood samples were added to 2 ml of 6% (w/v) HClO₄ and the mixtures centrifuged in an M.S.E. Chilspin centrifuge at 3000 rev./min for 10 min to remove protein. Radioactivity was determined in the supernatant (0.2 ml). At 20 min after injection, samples of inguinal mammary gland and liver were rapidly cut free and clamped between tongs cooled in liquid N₂ (Wollenberger *et al.*, 1960). The freeze-clamped tissue was powdered in liquid N₂ with a pestle and mortar, and a portion of the frozen powder (about 1 g) was extracted with 4 ml of 6% (w/v) HClO₄ by homogenization with a motor-driven Teflon homogenizer. This homogenate (1 ml) was centrifuged at 3000 rev./min for 10 min and the pellet was extracted with 5 ml of chloroform/methanol (2:1, v/v). Radioactivity in this extract was taken to be a measure of the conversion of

[U-¹⁴C]leucine into lipid. The defatted residue was dried at 60–70°C and 1 ml of Soluene 350 was used to solubilize the residue and measure the conversion of [U-¹⁴C]leucine into protein. Arterial blood was collected from the aorta into a heparinized syringe for measurements of L-leucine concentration with an amino acid analyser.

Studies in vitro: preparation of acini. Acini were prepared by a modification of the procedure of Katz *et al.* (1974). For full details, see Robinson & Williamson (1977a) and Williamson & Robinson (1979).

Incubation procedure. The acini (about 100 mg wet wt.) were incubated in a total volume of 4 ml of Krebs–Henseleit (1932) saline containing glucose (5 mM) and other additions where indicated. The following concentrations of other compounds were used where stated: leucine (1 mM), acetoacetate (2 mM), (–)-hydroxycitrate (5 mM) and insulin (30 munits/ml). Measurements of the conversion of radioactive compounds into lipids were made as described previously (Robinson & Williamson, 1977a).

Determination of metabolites. The metabolites were determined by enzymic methods: D-glucose (Slein, 1963), L-lactate and pyruvate (Hohorst *et al.*, 1959). The amino acid concentrations in blood were determined with a JEOL JLC-6AH amino acid analyser.

Measurements of radioactivity. These were carried out as described by Williamson *et al.* (1975).

Expression of results

Rates of glucose utilization, lactate and pyruvate production, and ¹⁴C incorporation into CO₂, protein and lipid by acini were obtained from graphical plots of the 20, 40 and 60 min values. The results are expressed as μ mol/min per 100 mg defatted dry wt. The half-life ($t_{0.5}$) of [1-¹⁴C]leucine and [U-¹⁴C]leucine was calculated from the semilogarithmic plots of the value for blood radioactivity at 1, 2, 3, 4 and 5 min; these were linear. Incorporation of radioactivity into lipids and proteins of mammary gland and liver is expressed as % of dose/g wet wt. of tissue at 20 min after injection.

Results and discussion

Metabolism of L-leucine in vivo

At peak lactation the dietary intake of the rat is increased about 3-fold (Fell *et al.*, 1963), but this was associated with a decrease in the concentration of leucine in whole blood [0.135 ± 0.015 (6) μ mol/ml in virgin rats compared with 0.107 ± 0.028 (16) μ mol/ml in lactating rats: $P < 0.05$]. This suggested that the utilization of the amino acid might be increased in lactation, and this has been confirmed by measurement of the half-life ($t_{0.5}$) and turnover of

L-[1-¹⁴C]leucine (Table 1). The $t_{0.5}$ decreased by 33% and the turnover rate increased by 35% in lactating rats compared with virgin rats. Starvation of virgin rats also resulted in a slight decrease in the blood leucine concentration, but this was accompanied by a 37% decrease in turnover rate. This latter finding is not in agreement with the report that the production of ¹⁴CO₂ from L-[1-¹⁴C]leucine is increased in starved rats (Meikle & Klain, 1972). It must be pointed out, however, that in this latter study the rats were starved for 4 days. Leucine removal is decreased in man on starvation or dietary restriction (Sherwin, 1978; Gelfand *et al.*, 1979; Elia *et al.*, 1980). Inhibition of lactation by either starvation or removal of the pups caused no change in the blood concentrations of leucine, but the turnover rates declined to the value for the fed virgin rats, though they were still higher than that for starved virgin rats (Table 1). Similar values for $t_{0.5}$ were obtained with [U-¹⁴C]leucine (results not shown).

The tissue likely to be responsible for the increased removal of leucine in the lactating rat is the mammary gland. To test this prediction the incorporation of L-[U-¹⁴C]leucine into lipid and protein of mammary gland and liver of virgin and lactating rats was measured (Table 2). Incorporation of L-[U-¹⁴C]leucine into lipid and protein of

mammary glands of the lactating rats increased 7-fold and 6-fold respectively compared with the glands from virgin rats. If one takes into account the increase in mammary-gland weight on transition from the virgin to the lactating state (approx. 4-fold; Chatwin *et al.*, 1969), it is clear that the lactating gland is a major site of leucine metabolism. Starvation (24 h) of lactating rats resulted in a considerably decreased incorporation of leucine into the lipid and protein of the gland, but the values were still significantly ($P < 0.05$) higher than those for virgin rats (Table 2). There was no significant alteration in the amount of leucine incorporation into hepatic lipid and protein during lactation (Table 2).

Metabolism of [1-¹⁴C]leucine in isolated acini

The first irreversible step in leucine catabolism is the reaction catalysed by branched-chain oxo acid dehydrogenase (EC 1.2.3.4), which results in the formation of CO₂ from C-1 of leucine. Measurement of the rate of ¹⁴CO₂ produced from L-[1-¹⁴C]leucine therefore indicates the flux of the amino acid into the catabolic pathway (Table 3). In the absence of glucose in the medium, the rate of oxidation of L-[1-¹⁴C]leucine was 50% lower than when glucose is present (results not shown). This finding may be explained by a deficiency of oxo acids (mainly oxoglutarate) for transamination when

Table 1. Blood concentration, $t_{0.5}$, distribution space and turnover rates of L-[1-¹⁴C]leucine in virgin and lactating rats. For details see the Experimental section. The results are mean values $\pm$ S.D. with the numbers of observations in parentheses. Results which are significantly different from those for fed virgin rats are shown: * $P < 0.05$; ** $P < 0.005$.

State of rats	Blood L-leucine (μ mol/ml)	L-[1- ¹⁴ C]Leucine		
		$t_{0.5}$	Distribution space (ml/100 g body wt.)	Turnover rate (μ mol/h per 100 g body wt.)
Virgin, fed (5)	0.133 $\pm$ 0.015	157 $\pm$ 21	36.1 $\pm$ 7.9	56.3 $\pm$ 11.4
Virgin, starved (24 h) (4)	0.108 $\pm$ 0.023*	180 $\pm$ 50	29.7 $\pm$ 2.5	35.0 $\pm$ 6.7**
Lactating, fed (5)	0.110 $\pm$ 0.040	105 $\pm$ 15**	40.3 $\pm$ 5.8	75.9 $\pm$ 19.2*
Lactating, 'weaned' (4)	0.092 $\pm$ 0.012**	122 $\pm$ 31*	40.0 $\pm$ 3.4	57.6 $\pm$ 21.2
Lactating, starved (24 h) (4)	0.110 $\pm$ 0.025	135 $\pm$ 40	35.5 $\pm$ 5.8	56.1 $\pm$ 19.0

Table 2. Incorporation of L-[U-¹⁴C]leucine into lipid and protein of mammary gland and liver of virgin and lactating rats. For details see the Experimental section. Incorporation of radioactivity (20 min after injection) into lipid and protein of mammary gland and liver is expressed as % of dose/g wet wt. of tissue. The results are mean values $\pm$ S.D., with the numbers of observations in parentheses. Values which are significantly different from those for the virgin rats are shown: * $P < 0.05$; ** $P < 0.005$.

State of rats	Percentage of dose of ¹⁴ C incorporated into			
	Mammary gland		Liver	
	Lipid	Protein	Lipid	Protein
Virgin, fed (4)	0.027 $\pm$ 0.008	0.144 $\pm$ 0.039	0.086 $\pm$ 0.026	0.355 $\pm$ 0.114
Lactating, fed (5)	0.196 $\pm$ 0.091**	0.865 $\pm$ 0.272**	0.085 $\pm$ 0.028	0.351 $\pm$ 0.043
Lactating, starved (24 h) (5)	0.046 $\pm$ 0.016*	0.330 $\pm$ 0.080*	0.075 $\pm$ 0.039	0.500 $\pm$ 0.140

Table 3. *Oxidation of L-[1-¹⁴C]leucine to ¹⁴CO₂ in isolated acini from mammary glands of lactating rats*

For details see the Experimental section. The incubation medium contained L-[1-¹⁴C]leucine (1 mM), glucose (5 mM), sodium acetoacetate (2 mM) and insulin (30 munits/ml) where indicated. The results are mean values $\pm$ S.D., expressed as μ mol of L-[1-¹⁴C]leucine converted into ¹⁴CO₂/min per 100mg defatted dry wt., with the numbers of observations in parentheses. Values that are significantly different from those for the lactating rats fed chow are indicated by: **P* < 0.05; ****P* < 0.001.

State of rats	¹⁴ CO ₂ formed		
	Glucose	Glucose + insulin	Glucose + acetoacetate
Lactating, chow-fed	0.22 $\pm$ 0.06 (13)	0.21 $\pm$ 0.06 (9)	0.17 $\pm$ 0.05 (6)
Lactating, starved (24 h)	0.20 $\pm$ 0.06 (8)	0.18 $\pm$ 0.06 (8)	0.24 $\pm$ 0.07 (3)
Lactating, 'weaned' (24 h)	0.051 $\pm$ 0.001 (3)***	—	0.039 $\pm$ 0.004 (3)***
Lactating, cafeteria diet	0.15 $\pm$ 0.07 (8)*	0.13 $\pm$ 0.05 (4)*	—

Table 4. *Effects of insulin on the metabolism of L-[U-¹⁴C]leucine and L-[2-¹⁴C]leucine in isolated acini from mammary glands of lactating rats*

For details see the Experimental section. The incubation medium contained glucose (5 mM), L-[U-¹⁴C]- or [2-¹⁴C]-leucine (1 mM) and insulin (30 munits/ml) where indicated. The results are mean values $\pm$ S.D., expressed as μ mol of leucine converted into lipid or ¹⁴CO₂/min per 100mg defatted dry wt., with the numbers of observations in parentheses. Values that are significantly different from those for chow-fed lactating rats in the presence and absence of insulin are shown: **P* < 0.05; ***P* < 0.005.

State of rats	L-[U- ¹⁴ C]Leucine incorporated into Lipids		L-[2- ¹⁴ C]Leucine incorporated into			
			¹⁴ CO ₂		Lipids	
	No insulin	+Insulin	No insulin	+Insulin	No insulin	+Insulin
Lactating, chow-fed	0.12 $\pm$ 0.010 (10)	0.12 $\pm$ 0.030 (6)	0.023 $\pm$ 0.007 (3)	0.007 $\pm$ 0.007 (3)	0.17 $\pm$ 0.05 (3)	0.19 $\pm$ 0.05 (3)
Lactating, starved (24 h)	0.081 $\pm$ 0.033 (7)**	0.077 $\pm$ 0.022 (7)**	0.069 $\pm$ 0.019 (4)**	0.019 $\pm$ 0.010 (4)*	0.14 $\pm$ 0.04 (4)	0.16 $\pm$ 0.03 (4)
Lactating, cafeteria diet	0.078 $\pm$ 0.023 (12)**	0.12 $\pm$ 0.009 (12)	0.059 $\pm$ 0.017 (6)**	0.023 $\pm$ 0.002 (6)**	0.085 $\pm$ 0.014 (6)**	0.15 $\pm$ 0.019 (6)*

exogenous precursors are omitted from the medium. The rate of production of ¹⁴CO₂ (in the presence of glucose) was the same in acini isolated from glands of fed (chow diet) and starved (24 h with pups) lactating rats (Table 3). This suggests that the capacity of the branched-chain oxo acid dehydrogenase *in vitro* is unchanged in starvation, which contrasts with the inactivation of pyruvate dehydrogenase in mammary glands of starved lactating rats (Baxter & Coore, 1978). Feeding with a cafeteria diet (high in fat) did cause a small but significant (*P* < 0.05) decrease in ¹⁴CO₂ production. Removal of the pups for 24 h resulted in a 75% decrease in the oxidative decarboxylation of leucine, and this is presumably due to a loss of enzyme activity on involution of the mammary tissue. In adipose tissue insulin increases the production of ¹⁴CO₂ from L-[1-¹⁴C]leucine in the presence and absence of glucose (Goodman, 1977). Insulin had no significant effect on the oxidative decarboxylation of L-[1-¹⁴C]leucine, even in acini from lactating rats fed on a cafeteria diet, which show marked sensitivity of glucose metabolism to insulin (Table 6; Agius *et al.*,

1980). Acetoacetate (2 mM) inhibited production of ¹⁴CO₂ from L-[1-¹⁴C]leucine by 23% (*P* < 0.05) in acini from lactating rats fed on a chow diet and by 24% (*P* < 0.005) in acini from 'weaned' rats. This inhibition may be due to competition for the available CoA. In contrast, stimulation of oxidative decarboxylation of leucine by ketone bodies has been observed in muscle homogenates (Paul & Adibi, 1978). There was no effect of acetoacetate on leucine catabolism in acini from starved lactating rats.

Metabolism of L-[U-¹⁴C]- or [2-¹⁴C]-leucine in isolated acini

To obtain information on the conversion of leucine into lipid, acini were incubated with either L-[U-¹⁴C]- or [2-¹⁴C]-leucine (Table 4); the latter forms a molecule of acetyl-CoA labelled with ¹⁴C at C-2. Incorporation of L-[U-¹⁴C]leucine into lipid decreased by 33% in acini from starved lactating rats and by 35% in acini from lactating rats fed on a cafeteria diet (Table 4). Insulin had no significant effect on the incorporation of L-[U-¹⁴C]leucine into

lipid in acini from lactating rats fed on a chow diet or in acini from starved lactating rats, but it stimulated conversion of leucine into lipid in acini from lactating rats fed on a cafeteria diet by 35% ($P < 0.005$; Table 4).

Oxidation of L-[2- ^{14}C]leucine to $^{14}\text{CO}_2$ was increased about 3-fold in acini from starved lactating rats or lactating rats fed on a cafeteria diet; insulin decreased the formation of $^{14}\text{CO}_2$ in all three situations studied (Table 4). The changes in incorporation of L-[2- ^{14}C]leucine into lipid paralleled those with L-[U- ^{14}C]leucine, except that the decreased incorporation in acini from starved lactating rats was not significant. Insulin tended to increase the rate of L-[2- ^{14}C]leucine incorporation into lipid in acini from lactating rats fed on a chow diet and in acini from starved lactating rats, but the changes were not significant. However, insulin stimulated the depressed rate of lipogenesis in acini from lactating rats fed on a cafeteria diet by 76% ($P < 0.005$; Table 4). In adipose tissue insulin also decreases the oxidation of L-[2- ^{14}C]leucine to $^{14}\text{CO}_2$ and increases its incorporation into lipid (Goodman, 1977).

Under these experimental conditions (no other added amino acids in the medium), only 15% of the [U- ^{14}C]leucine utilized was incorporated into protein in acini from fed or starved lactating rats (results not shown).

Route for conversion of leucine into lipid

Leucine is metabolized to acetoacetate and acetyl-CoA within the mitochondria. Conversion of these products into lipid could occur by at least two routes: (1) the acetyl-CoA might condense with oxaloacetate to form citrate, and this can then be transported out of the mitochondria to the cytosol

for reconversion into acetyl-CoA; (2) alternatively, the acetoacetate might be transported to the cytosol (instead of being converted into acetyl-CoA), where it could react with acetoacetyl-CoA synthetase and acetoacetyl-CoA thiolase to provide acetyl-CoA directly for lipogenesis (Buckley & Williamson, 1973). Some indirect evidence for the operation of this latter pathway has been presented for rat brain (Patel & Owen, 1976) and for mouse liver and adipocytes (Rous & Favarger, 1973). We decided to test the relative importance of the two routes by use of an inhibitor of citrate cleavage enzyme, namely (–)-hydroxycitrate (Watson *et al.*, 1969), which should allow the pathway via acetoacetate efflux from the mitochondria to remain active.

(–)-Hydroxycitrate (5 mM) decreased [1- ^{14}C]glucose conversion into lipid by 60%, but at the same time it decreased glucose removal by 42% (Table 5). The inhibitor decreased the formation of lipid from [U- ^{14}C]leucine by only 40%, whereas the removal of glucose in the presence of leucine was depressed to a similar extent (39%) as in its absence. These results can be interpreted as indicating that the major pathway for the conversion of leucine carbon into lipid is via citrate translocation, but a contribution by efflux of acetoacetate to the cytosol is not excluded. It must be emphasized that in the presence of (–)-hydroxycitrate glucose utilization and therefore availability of NADPH generated via the pentose phosphate pathway and of glycerol 3-phosphate for esterification are depressed; these changes may have a direct effect on lipogenesis irrespective of the pathway of carbon flux. Presumably, (–)-hydroxycitrate increases the concentration of citrate in cytosol, which in turn inhibits mammary-gland phosphofructokinase (Zammit, 1979) and decreases glucose utilization. We con-

Table 5. Effects of (–)-hydroxycitrate on [1- ^{14}C]glucose and [U- ^{14}C]leucine metabolism in isolated acini from mammary glands of fed lactating rats

For details see the Experimental section. The incubation medium contained glucose (5 mM), L-leucine (1 mM) and (–)-hydroxycitrate (5 mM). The results are mean values $\pm$ S.D. expressed as $\mu\text{mol/min}$ per 100 mg defatted dry wt. for glucose, lactate and pyruvate, and as $\mu\text{mol/min}$ per 100 mg defatted dry wt. for [1- ^{14}C]glucose or [U- ^{14}C]leucine converted into $^{14}\text{CO}_2$ or [^{14}C]lipids, with the numbers of observations in parentheses. Values that are significantly different from the incubation with [1- ^{14}C]glucose in the absence of (–)-hydroxycitrate are shown: * $P < 0.05$; ** $P < 0.005$. Those that are different from the incubation with glucose + [U- ^{14}C]leucine are shown: † $P < 0.05$; †† $P < 0.005$.

Additions	Glucose removed	$^{14}\text{CO}_2$ formed	[^{14}C]Lipids formed	Lactate formed	Pyruvate formed
[1- ^{14}C]Glucose	1.37 $\pm$ 0.26 (7)	0.53 $\pm$ 0.12 (7)	0.32 $\pm$ 0.09 (7)	0.48 $\pm$ 0.17 (7)	0.096 $\pm$ 0.02 (7)
[1- ^{14}C]Glucose + (–)-hydroxycitrate	0.79 $\pm$ 0.25* (3)	0.24 $\pm$ 0.07** (3)	0.13 $\pm$ 0.05* (3)	0.26 $\pm$ 0.05* (3)	0.040 $\pm$ 0.02** (3)
Glucose + [U- ^{14}C]leucine	1.18 $\pm$ 0.27 (19)	—	0.12 $\pm$ 0.01 (10)	0.38 $\pm$ 0.17 (19)	0.15 $\pm$ 0.07 (19)
Glucose + [U- ^{14}C]leucine + (–)-hydroxycitrate	0.72 $\pm$ 0.29†† (5)	—	0.070 $\pm$ 0.015†† (5)	0.25 $\pm$ 0.07† (5)	0.086 $\pm$ 0.05† (5)

clude that use of (—)-hydroxycitrate to test for the proportion of lipid formed by citrate efflux from the mitochondria is open to question if, as in the present experiments, the rate of glucose utilization is altered by the inhibitor.

Effects of L-leucine on glucose metabolism in isolated acini

Acetoacetate at physiological concentrations inhibits glucose removal by isolated acini (Robinson & Williamson, 1977b), and therefore the effects of leucine (a precursor of acetoacetate) on glucose metabolism in acini were examined. Leucine had no significant effect on glucose removal by acini from mammary glands of lactating rats fed on a chow or cafeteria diet or from starved lactating rats (Table 6). However, leucine did significantly ($P < 0.05$) increase the accumulation of pyruvate in the medium in all situations except acini from lactating rats fed on a chow diet and incubated with glucose and insulin (Table 6). The accumulation of lactate in the medium was not altered significantly by leucine, except for acini from starved lactating rats incubated with glucose, where there was a 40% decrease. The net result of these changes was a decrease in the lactate/pyruvate ratio in the medium in the presence of leucine. Possible reasons for the accumulation of pyruvate in the presence of leucine are: (1) competition between pyruvate and 4-methyl-2-oxopentanoate for transport into the mitochondria (Halestrap *et al.*, 1974) and (2) competition between the branched-chain oxo acid dehydrogenase and pyruvate dehydrogenase for NAD and CoA.

Physiological implications

In non-lactating rats the major sites of leucine removal are skeletal muscle and adipose tissue. With the increase in dietary intake of the lactating rat more leucine is available, and the present results indicate that the mammary gland becomes a major site of leucine removal. A supply of leucine is required by the gland for casein synthesis, and it appears that leucine can also act as an effective precursor of lipid in the gland.

As might be expected from a physiological standpoint, the turnover rate of leucine is decreased in the starved lactating rat, as is its conversion into lipid and protein in mammary gland. Surprisingly, the experiments *in vitro* indicate no change in the oxidative decarboxylation of leucine on transition from the fed to the starved state in isolated acini, unlike the marked inhibition of pyruvate oxidation. This apparent anomaly between the findings *in vivo* and *in vitro* might be explained by a decrease in starvation in blood flow to the gland and/or an increase in the complete oxidation of leucine.

Table 6. *Effects of L-leucine on glucose metabolism in isolated acini from mammary glands of lactating rats*

For details see the Experimental section. The incubation medium contained glucose (5 mM), L-leucine (1 mM) and insulin (30 munits/ml) where indicated. The results are mean values $\pm$ S.D., expressed as $\mu\text{mol/min per } 100 \text{ mg defatted dry wt.}$, with the numbers of observations in parentheses. Values which are significantly different from the incubation with glucose alone are shown: * $P < 0.05$; ** $P < 0.005$.

Additions	Lactating, fed on chow diet			Lactating, starved (24 h)			Lactating, fed on cafeteria diet		
	Glucose removed	Lactate formed	Pyruvate formed	Glucose removed	Lactate formed	Pyruvate formed	Glucose removed	Lactate formed	Pyruvate formed
Glucose	1.35 $\pm$ 0.32 (12)	0.45 $\pm$ 0.24 (12)	0.11 $\pm$ 0.06 (12)	0.94 $\pm$ 0.40 (9)	1.20 $\pm$ 0.45 (9)	0.18 $\pm$ 0.07 (9)	0.75 $\pm$ 0.24 (10)	0.45 $\pm$ 0.15 (10)	0.058 $\pm$ 0.019 (10)
Glucose + insulin	—	—	—	—	—	—	1.34 $\pm$ 0.53** (9)	0.67 $\pm$ 0.21* (9)	0.076 $\pm$ 0.026* (9)
Glucose + leucine	1.18 $\pm$ 0.27 (19)	0.38 $\pm$ 0.17 (19)	0.15 $\pm$ 0.07* (19)	0.87 $\pm$ 0.30 (18)	0.71 $\pm$ 0.27** (18)	0.23 $\pm$ 0.08* (18)	0.80 $\pm$ 0.24 (26)	0.37 $\pm$ 0.17 (22)	0.094 $\pm$ 0.041* (22)
Glucose + leucine + insulin	1.55 $\pm$ 0.41 (16)	0.40 $\pm$ 0.19 (16)	0.10 $\pm$ 0.06 (16)	1.17 $\pm$ 0.32* (18)	0.98 $\pm$ 0.36 (18)	0.25 $\pm$ 0.10* (18)	1.36 $\pm$ 0.32** (22)	0.61 $\pm$ 0.23* (20)	0.136 $\pm$ 0.051** (20)

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