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Effects of Acetoacetate Administration on Glucose Metabolism in Mammary Gland of Fed Lactating Rats

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Administration of acetoacetate to fed lactating rats rapidly decreases glucose uptake by the mammary gland, and causes an output of pyruvate, whereas lactate uptake remains unchanged. Similar changes, though not identical, occur in starved lactating rats, which suggests that the increased acetoacetate concentration in this situation may be one of the factors responsible for the alterations in glucose metabolism.

Arteriovenous-difference measurements across the mammary gland of lactating rats have indicated that in short-term starvation (16–24h) glucose uptake by the gland is decreased, ketone bodies are taken up and lactate and pyruvate are released rather than being extracted (Hawkins & Williamson, 1972; Robinson & Williamson, 1977). Studies *in vitro* with mammary-gland slices have shown that physiological concentrations of acetoacetate decrease glucose utilization and that this effect can be reversed by insulin (Williamson *et al.*, 1975). This has led to the proposal that the changes in glucose metabolism in starvation might be brought about by a lower concentration of circulating insulin and/or increased concentrations of ketone bodies (Williamson *et al.*, 1975). To test this suggestion measurements have been made of arteriovenous differences and metabolite concentrations in freeze-clamped glands from fed rats after acetoacetate injection.

Experimental

Rats of the Wistar strain (280–340g) with between six and fourteen pups were used after a lactating period of between 10 and 18 days. The rats were anaesthetized with Nembutal [50mg/kg body wt., in saline (0.9% NaCl)] and kept with their litters for about 5min after onset of anaesthesia. A sample of venous blood was collected from the left pudic-epigastric vein into a heparinized syringe with a 25-gauge needle. The inguinal mammary gland on the same side was then freed from surrounding tissue and rapidly cut free and clamped between tongs cooled in liquid N_2 (Wollenberger *et al.*, 1960). Then acetoacetate (0.5ml of a 1M solution) or saline (0.5ml) was injected into the tail vein. After 5min a sample of venous blood was collected from the right pudic-epigastric vein and a piece of the inguinal mammary gland on this side was freeze-clamped. A sample of arterial blood was then immediately collected from the aorta.

The blood samples (0.5ml) and the freeze-clamped tissue (about 1g) were treated as previously described (Robinson & Williamson, 1977). The following metabolites were determined in the neutralized $HClO_4$ extracts by enzymic methods: glucose (Slein, 1963); L-lactate and pyruvate (Hohorst *et al.*, 1959); acetoacetate and D-3-hydroxybutyrate (Williamson *et al.*, 1962); glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963); AMP and ADP (Adam, 1963); L-glycerol 3-phosphate (Hohorst, 1963).

The experimental design provides two methods for assessing the effects of the acetoacetate injection. The samples of venous blood and freeze-clamped gland taken after injection were compared (Student's *t* test for paired observations) with the samples of venous blood and gland taken before injection. In addition, the mean concentrations of metabolites in the arterial and venous blood and the freeze-clamped gland after injection of acetoacetate or saline were compared (Student's *t* test).

Results and Discussion

The concentrations of metabolites in the venous blood taken before injection of acetoacetate or saline (Table 1) did not differ significantly from the venous concentrations found previously in this laboratory for fed lactating rats (Robinson & Williamson, 1977). After saline injection there were no significant changes in the venous blood concentrations of lactate, pyruvate or ketone bodies, but the glucose concentration decreased ($P<0.05$; Table 1). Acetoacetate injection increased the blood concentration of ketone bodies to 1.6mM and this was accompanied by a 2-fold increase in the concentration of pyruvate. The arterial blood glucose concentration is not decreased by 24h of starvation (Robinson & Williamson, 1977), but after the acetoacetate injection the arterial blood glucose concentration of the

Table 1. *Concentrations of metabolites in blood and arteriovenous differences across inguinal mammary gland of fed lactating rats after acetoacetate or saline injection*

The results are mean values ± S.D. expressed as μmol/ml of whole blood. The numbers of rats are shown in parentheses. Venous blood samples (*a*) were taken from the pudic-epigastric vein before injection. Venous samples (*b*) and arterial samples were collected after injection as described in the Experimental section. Arteriovenous differences were calculated from arterial and venous blood concentrations after injection. The symbols + and − indicate production or uptake of the metabolite respectively. Concentrations that are statistically different (Student's *t* test for paired observations) in venous (*a*) and venous (*b*) samples have: †$P<0.025$, ††$P<0.0005$. Values that are statistically different (Student's *t* test) after acetoacetate injection compared with saline injection have: *$P<0.01$, **$P<0.0005$.

Injection given	Blood	Metabolite concn. (μmol/ml of whole blood)				
		Glucose	Lactate	Pyruvate	Acetoacetate	3-Hydroxybutyrate
Acetoacetate (6)	Venous (*a*)	3.04 ± 0.60	1.06 ± 0.36	0.12 ± 0.05	0.06 ± 0.03	0.06 ± 0.04
	Venous (*b*)	2.90 ± 0.85	1.18 ± 0.15*	0.23 ± 0.04**††	0.94 ± 0.15**††	0.62 ± 0.13**††
	Arterial	3.77 ± 0.72	1.70 ± 0.29	0.15 ± 0.03*	0.92 ± 0.22**	0.69 ± 0.07**
	Difference	−0.87 ± 0.31**	−0.52 ± 0.25	+0.08 ± 0.04**	+0.02 ± 0.19	−0.07 ± 0.11
Saline (6)	Venous (*a*)	3.20 ± 1.09	1.00 ± 0.41	0.08 ± 0.04	0.04 ± 0.01	0.05 ± 0.01
	Venous (*b*)	2.68 ± 0.54	0.79 ± 0.15	0.07 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
	Arterial	4.62 ± 0.60	1.61 ± 0.46	0.09 ± 0.03	0.06 ± 0.02	0.09 ± 0.03
	Difference	−1.94 ± 0.41	−0.82 ± 0.37	−0.02 ± 0.03	−0.03 ± 0.01	−0.05 ± 0.02

fed lactating rats was significantly lower than both the arterial blood glucose concentration after saline injection ($P<0.05$; Table 1) and the arterial blood glucose concentration of 4.74 ± 0.48 μmol/ml found previously ($P<0.01$; Robinson & Williamson, 1977). This decrease in blood glucose could well be due to a stimulation of insulin secretion by the increase in blood ketone-body concentrations (Hawkins *et al.*, 1971).

Glucose uptake across the gland was decreased after acetoacetate injection compared with the saline-injected controls (Table 1) and with the glucose uptake of 1.43 ± 0.47 μmol/ml of whole blood found previously for fed lactating rats (Robinson & Williamson, 1977). This low glucose uptake is similar to that found for starved (24 h) lactating rats, where the ketone body concentration in the arterial blood was 0.54 mM (Robinson & Williamson, 1977), and suggests a direct interaction between glucose and acetoacetate metabolism in the fed rat, although the concentrations of ketone bodies achieved in the present experiments were 3-fold higher than in the starved lactating rats. However, it would not be unexpected to find that higher acetoacetate concentrations are required in fed rats to obtain an appreciable effect on glucose utilization because fed lactating rats have higher plasma insulin concentrations than starved lactating rats (J. P. Girard, A. M. Robinson & D. H. Williamson, unpublished work) and insulin relieves the inhibition of glucose utilization by acetoacetate *in vitro* (Williamson *et al.*, 1975; A. M. Robinson & D. H. Williamson, unpublished work). Alternative explanations for the apparent decrease in glucose uptake are the lower arterial glucose concentrations of an increase in mammary-gland blood flow. There appears to be no correlation, however, between the individual arterial glucose concentrations and the arteriovenous differences for glucose across the gland in our present experiments or those published previously (Robinson & Williamson, 1977), suggesting that the lower arterial gluocse concentration is not the reason for the decreased uptake of glucose on injection of acetoacetate.

In starvation the increased ketone-body concentration in the blood is accompanied by an increased uptake by the mammary gland (Hawkins & Williamson, 1972; Robinson & Williamson, 1977), but in the present experiments the arteriovenous difference for ketone bodies across the mammary gland is not increased after acetoacetate injection (Table 1). This may be explained by the rapid decrease in the arterial concentration of ketone bodies after acetoacetate injection, due to utilization by rat tissues, which causes the measured arterial concentration of ketone bodies (Table 2) to be lower than their actual concentrations at the instant of time when the second venous blood sample was obtained.

Increased ketone-body concentrations did not decrease lactate uptake by the glands of lactating rats, but pyruvate was released from the gland rather than being taken up as in the saline-injected rats (Table 1). The uptake of lactate in the acetoacetate-injected rats contrasts with the release of lactate from the glands of starved (16–24 h) lactating rats (Hawkins & Williamson, 1972; Robinson & Williamson, 1977).

In the samples of freeze-clamped gland obtained before injection there were few significant differences between the metabolite concentrations of the two experimental groups (Table 2), and the metabolite concentrations were similar to those found previously for glands from fed lactating rats (Robinson &

Table 2. *Concentrations of metabolites in freeze-clamped mammary gland of fed lactating rats before and after acetoacetate or saline injection*

The results are mean values ± S.D. expressed as μmol/g wet wt. of gland. The numbers of rats are shown in parentheses. Concentrations were measured in freeze-clamped mammary gland removed before (*a*) and after (*b*) injection as described in the Experimental section. Concentrations before injection (*a*) that are statistically different (Student's *t* test for paired observations) to concentrations after injection (*b*) have: †$P<0.025$, ††$P<0.0005$. Values that are statistically different (Student's *t* test) after acetoacetate injection compared with after saline injection have: *$P<0.01$; **$P<0.0005$.

	Metabolite concn. (μmol/g wet wt.)			
	Injection given ... Acetoacetate		Saline	
Metabolites	Before (*a*)	After (*b*)	Before (*a*)	After (*b*)
Glucose	1.23 ± 0.83 (6)	1.17 ± 0.62	0.99 ± 0.48 (6)	1.11 ± 0.69
Lactate	1.64 ± 0.56 (6)	1.80 ± 0.64	1.67 ± 1.04 (6)	1.19 ± 0.48
Pyruvate	0.11 ± 0.05 (6)	0.31 ± 0.05**††	0.07 ± 0.01 (6)	0.08 ± 0.02
Acetoacetate	0.05 ± 0.02 (6)	0.35 ± 0.20*†	0.02 ± 0.01 (6)	0.03 ± 0.01
3-Hydroxybutyrate	0.04 ± 0.01 (5)	0.41 ± 0.15**††	0.04 ± 0.01 (6)	0.03 ± 0.01†
Glycerol 3-phosphate	0.19 ± 0.07 (6)	0.12 ± 0.05*†	0.22 ± 0.04 (6)	0.22 ± 0.04
Citrate	0.26 ± 0.04 (6)	0.39 ± 0.07*†	0.22 ± 0.02 (6)	0.24 ± 0.05
2-Oxoglutarate	0.05 ± 0.01 (5)*	0.10 ± 0.02**††	0.03 ± 0.01 (6)	0.05 ± 0.01
Malate	0.26 ± 0.06 (4)	0.35 ± 0.06*	0.21 ± 0.06 (6)	0.19 ± 0.04
Glucose 6-phosphate	0.13 ± 0.03 (5)	0.11 ± 0.03	—	—
ATP	1.45 ± 0.19 (5)	1.25 ± 0.18	—	—

Williamson, 1977). After administration of acetoacetate the tissue [acetoacetate] and [3-hydroxybutyrate] were increased in the gland, but there was no change in [glucose] or [lactate]. Injection of acetoacetate increased [pyruvate], [2-oxoglutarate], [citrate] and [malate] by 280, 200, 150 and 135% respectively, while [glycerol 3-phosphate] was decreased by 50% (Table 2). There was no significant change in [glucose 6-phosphate], [ATP], [ADP] and [AMP] with acetoacetate injection (Table 2; values for [ADP] and [AMP] are not shown).

It has been suggested that acetoacetate inhibition of glucose uptake in slices of lactating rat mammary gland involves inhibition of phosphofructokinase by increased [citrate], resulting in increased [glucose 6-phosphate], which in turn inhibits hexokinase (Williamson *et al.*, 1975). The increased [citrate] in the gland after acetoacetate injection is consistent with this hypothesis, although the fact that [glucose 6-phosphate] was unchanged is not.

Changes in the redox state of the tissue due to ketone-body utilization may have contributed to the alterations in mammary-gland metabolism in acetoacetate-injected rats. After acetoacetate injection the [lactate]/[pyruvate] ratio in the gland decreased from 15.3 to 5.8, which suggests that the cytoplasmic $[NAD^+]/[NADH]$ ratio is increased. Likewise, the [lactate]/[pyruvate] ratio decreases in incubations of rat mammary-gland slices with glucose and acetoacetate (Williamson *et al.*, 1975). The decreased [glycerol 3-phosphate] in the gland after acetoacetate injection may similarly reflect decreased availability of cytosolic NADH (Kuhn, 1967). In acetoacetate-injected rats the [3-hydroxybutyrate]/[acetoacetate] ratio was 1.2 in the mammary gland (Table 2) and 0.7 in the venous blood (Table 1). The higher ratio in the gland than in the venous blood and the apparent uptake of 3-hydroxybutyrate suggests that conversion of 3-hydroxybutyrate into acetoacetate was occurring in the gland. Pyruvate concentrations may increase in the mammary gland of acetoacetate-injected rats because pyruvate dehydrogenase is inactivated by both a decreased mitochondrial $[NAD^+]/[NADH]$ ratio and increased [acetyl-CoA] (Denton *et al.*, 1975), which may result from the increased utilization of ketone bodies in the mammary gland.

The metabolic changes resulting from starvation are retained in acini isolated from mammary glands of starved rats (Robinson & Williamson, 1977), whereas the effects of acetoacetate injection in fed rats appear to be reversible. Thus acini prepared from mammary glands of fed rats that had been injected with acetoacetate and killed after 10 min did not show a decreased glucose uptake or increased lactate plus pyruvate production (A. M. Robinson & D. H. Williamson, unpublished work).

The present findings indicate that administration of acetoacetate to a fed rat results in changes in mammary-gland metabolism that are similar to, but not identical with, those occurring after 24h starvation of lactating rats. After acetoacetate administration or starvation glucose uptake is decreased and pyruvate is released by the gland. However, in

contrast with 24h starvation, where lactate is released by the mammary gland, after acetoacetate administration to fed rats lactate continues to be taken up by the gland. This difference may reflect longer-term changes in mammary-gland metabolism in starvation that may or may not be directly related to increased [ketone bodies]. One such change is the inactivation of mammary-gland pyruvate dehydrogenase on starvation (Kankel & Reinauer, 1976), which appears not to be easily reversed *in vitro* (Robinson & Williamson, 1977).

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