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Coordination of mammary metabolism and blood flow after refeeding in rats

K. W. Stewart,* G. J. S. Cooper,† and S. R. Davis‡
*School of Science and Primary Industries, Waikato Institute of Technology, Hamilton, New Zealand
†School of Biological Sciences, University of Auckland, New Zealand
‡Vialactia Biosciences, Hamilton, New Zealand

ABSTRACT

The production of milk is closely linked to nutritional state in many mammalian species, but the mechanisms by which changes in nutritional state are signaled to the mammary glands are poorly understood. Simultaneous measurements of mammary blood flow and glucose arterio-venous difference were made across the inguinal mammary glands of anesthetized, lactating rats. Blood flow to the mammary glands of previously fed rats was 0.48 mL/min per gram of mammary tissue. Glucose supply was 1.7 μmol/min per gram and 28% was extracted by the mammary glands. After food deprivation for 18 h, mammary blood flow decreased 48%, glucose arterio-venous difference decreased 72%, and hematocrit increased 7%, resulting in a 60% decrease in glucose supply and an 88% decrease in glucose uptake. After 1 h of refeeding, glucose supply had returned to a similar level to that of normally fed animals, but glucose uptake was 60% higher than in the normally fed state. Mammary glucose uptake was not closely linked to either blood flow or glucose supply, suggesting that substrate supply was not the primary determinant of mammary metabolism. Denervation experiments showed that the mammary metabolic response to altered nutritional state was also unlikely to be closely controlled by neural pathways. Severance of the cutaneous branch of the posterior division of the femoral nerve innervating the inguinal mammary glands did not reduce the high glucose uptake by mammary glands of either fed or refed rats, nor did denervation change the low glucose uptake by mammary glands of food-deprived rats. Denervation reduced blood flow in the associated mammary gland, however, indicating that neural pathways may play a role in supporting mammary blood flow when food is available. In in vitro experiments, the rate of glucose uptake was 35% lower in mammary acini from food-deprived rats than in fed rats 2.5 h after tissue removal, indicating some persistence of the food deprivation-induced suppression of mammary metabolism. Administration of insulin increased glucose uptake in acini from both fed and food-deprived rats, indicating that insulin may be involved in signaling the mammary gland of the restoration of nutrient supply when food-deprived rats are refed. The effects of administration of a gut extract in vivo and in vitro are discussed.

Key words: mammary blood flow, mammary glucose uptake, refeeding, mammary metabolism

INTRODUCTION

Milk production has been demonstrated to be acutely sensitive to availability of food in ruminants (Faulkner and Peaker, 1987) and rodents (Williamson and Robinson, 1977). For example, when food was removed from lactating rats, glucose extraction from mammary blood fell rapidly and substantially [60% after 6 h (Jones and Williamson, 1984) and 92% after 18 h (Page and Kuhn, 1986)]. In addition, mammary synthesis of both fatty acids and lactose fell to low levels after food withdrawal in lactating rats (Bussmann et al., 1984).

Mammary metabolism was rapidly restored to fed levels when food was reoffered to food-deprived rats. Mammary glucose extraction was largely restored in conscious lactating rats after only 15 min of refeeding and fully restored after 1 h (Page and Kuhn, 1986). In other studies, refeeding increased the uptake of 2-deoxy-D-glucose (**2DG**) by mammary glands to the fed level after 1 h (Threadgold and Kuhn, 1984) and restored glucose extraction to fed levels after 2 h (Jones and Williamson, 1984).

The pathways by which nutritional status is signaled to the mammary glands and the metabolic sites targeted by these pathways have not been clearly identified. This investigation examines aspects of the signaling pathways that lead to this rapid switching on of mammary metabolism on refeeding of food-deprived rats.

Insulin has been implicated in the mammary response to refeeding as it has been shown to influence mammary responses to nutritional changes. If insulin secretion was prevented, mammary lipogenesis did

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[1]Corresponding author: Kevin.Stewart@wintec.ac.nz

not show the usual recovery when food-deprived rats were refed (Mercer and Williamson, 1986). Conversely, insulin administration to food-deprived rats increased lipogenesis (Jones et al., 1984) and glucose extraction (Page and Kuhn, 1986). Furthermore, in studies in which food-deprived rats were refed, the time course of the recovery in lipogenesis (Mercer and Williamson, 1986) and glucose extraction (Page and Kuhn, 1986) was paralleled by that of blood insulin concentration.

Some researchers have speculated that a gut hormone may be involved in the increase in mammary metabolism observed after refeeding of food-deprived rats (Carrick and Kuhn, 1978; Williamson, 1980). It was suggested that this hormone is released when food becomes available or is ingested; hence, signaling the mammary glands of the end of the starvation state and activating metabolism. Such a mechanism was supported by Page (1989), who reported that intraperitoneal administration of a crude gut extract increased glucose extraction from blood supplying the inguinal mammary glands of anesthetized lactating rats if the cutaneous branch of the posterior division of the femoral nerve innervating the inguinal mammary glands was severed and blood insulin concentration was increased.

These 3 studies suggest that the physiological pathway controlling the increase in mammary metabolism on refeeding may be mediated by the coordinated involvement of a gut hormone, insulin, and neural pathways. However, we believe some caution should be applied to the interpretation of these results as we consider that the methods used to assess in vivo mammary glucose uptake in previous studies had inherent inaccuracies.

To accurately measure net glucose uptake rates, simultaneous measurements of mammary blood flow (**MBF**) and glucose arterio-venous difference (**AVD**) must be made under steady-state conditions (Zierler, 1961). This has not been achieved simultaneously in any published studies using rats. Mammary blood flow has not been directly measured in rats but has been estimated using less-accurate single measurement techniques such as i.v. injection of radioactive compounds [e.g., $^{3}H_2O$ (Chatwin et al., 1969) or ^{86}RbCl (Hanwell and Linzell, 1973)], microspheres (Jones and Williamson, 1984), or radiolabeled microspheres (Vina et al., 1987) and examination of their vascular distribution after a short period.

The methods employed for removal of mammary venous blood in previous studies was also likely to produce systematic errors. Blood was obtained either by puncturing the mammary vein and collecting blood by capillary action using a micro-hematocrit tube (Page and Kuhn, 1986; Page, 1989) or by aspiration using a syringe and 25-gauge needle introduced into the mammary vein (Hawkins and Williamson, 1972; Robinson and Williamson, 1977b; Vina et al., 1981, 1983). Further escape of blood was then prevented using pressure or by placing a piece of gauze over the hole in the vessel. Such procedures are likely to have altered MBF directly by obstructing venous drainage, and indirectly by inducing spasm of the mammary artery and vein (Mendelson and Scow, 1972; Clegg, 1988). Another potential problem with the removal of blood from the mammary vein using these methods is that mammary venous blood may be inadvertently contaminated by blood from veins distal to the mammary vein. Variation in blood flow produced by blood sampling procedures may explain the wide variation in reported results for glucose extraction by the inguinal mammary glands of fed rats at peak lactation [25% (Hawkins and Williamson, 1972); 30% (Robinson and Williamson, 1977a); 35% (Jones and Williamson, 1984); and 60% (Page and Kuhn, 1986)].

In the present study, a method was developed to make accurate measurements of glucose uptake in anesthetized rats to investigate the possible coordination of blood glucose and insulin levels with neural pathways and a factor from the gut in signaling the presence of food to the mammary glands. A rat mammary acini model was also used in vitro to assess cell performance after feed withdrawal and in response to hormonal stimulation.

MATERIALS AND METHODS

General Methods

This study was undertaken with the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand). Lactating Sprague-Dawley rats between d 12 and 16 postpartum were anesthetized with Nembutal [45 mg/kg intraperitoneal (**i.p.**) injection followed by 0.2 mg/kg per min i.p. infusion] and positioned dorsally on a platform maintained at 37°C. The rats were surgically prepared for measurement of blood flow to the left inguinal mammary glands. An incision was made through the skin on the inner surface of the left hind leg and the mammary tissue was lifted and separated from the underlying tissue to enable access to the iliac artery and vein. Under microscopy, all distal arteries apart from the inguinal mammary (superficial epigastric) artery were ligated. The iliac artery was separated from the associated vein, nerve, and other tissue to enable positioning of a perivascular flow probe (1 mm, Transonics Systems, Ithaca, NY). An acoustic coupling gel was applied to the probe as per manufacturer instructions and the probe carefully positioned to prevent disturbance of blood flow in the artery. Mammary blood flow was monitored continuously throughout each