Effects of dietary manipulations and glucose infusion on glucagon response during exercise in rats

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Effects of dietary manipulations and glucose infusion on glucagon response during exercise in rats. J. Appl. Physiol. 83(1): 148–152, 1997.—The purpose of the present investigation was to test the hypothesis that blood glucose concentration is not always related to glucagon response during exercise. Three groups of rats were submitted to a prolonged (3-h) swimming exercise. Two groups of rats had their normal food intake restricted by 50% the night before the experiment. One of these two groups of rats was intravenously infused with glucose throughout exercise to maintain euglycemia. The third group of rats swam while under normal dietary conditions. Plasma glucose, sampled in arterial blood, was reduced (P < 0.05) at 75, 105, 150, and 170 min of exercise (from 130 to 110 mg/dl) in the food-restricted animals without glucose infusion, whereas a significant (P < 0.05) increase was measured in the two other groups during exercise. A significant (P < 0.01) difference in the mean integrated areas under the glucose-concentration curve was found only between the fed and the two food-restricted groups. Plasma insulin concentrations decreased (P < 0.05) similarly in all groups during exercise, whereas plasma epinephrine and norepinephrine concentrations increased significantly (P < 0.01) in all groups. Despite differences between groups in plasma glucose response during exercise, and despite the absence of any decrease in exercising blood glucose levels in at least two of the three groups, plasma glucagon responses were increased (P < 0.05) similarly in all groups (from 250 to 550 pg/ml) at the end of the exercise period. The increase in glucagon was significant after 90 min of exercise in the food-restricted groups, with or without glucose infusion, but only after 140 min in the fed group. These results indicate that the glucagon response during exercise is not always linked to the decrease in plasma glucose.

hepatic glycogen; catecholamines; hypoglycemia

IT HAS BEEN REPORTED in numerous investigations that the liver, through its afferent innervation, is involved in the regulation of food intake (26, 27). The hepatic afferent branch of the vagus nerve has also been reported to participate in the regulation of insulin (17), glucagon (28), and epinephrine (4, 7, 14) secretion. These effects have been observed under different methodological approaches such as an acute hepatic vagotomy (17, 28), an insulin-induced hypoglycemia (4, 7, 14), and physical exercise (3, 15). The results of the above-mentioned studies are also in agreement with some electrophysiological evidence showing a neural link between the liver and the pancreas and between the liver and the adrenal glands (22).

The evidence of a link between the liver and the pancreas has brought us to question the possibility that an hepatic stimulus might influence the glucagon response during exercise. It is the general view that the glucagon response is associated with a decrease in plasma glucose. However, numerous physiological and pathological states such as liver cirrhosis (13, 20), hyperthyroidism (10), and partial hepatectomy (21) are characterized by hyperglucagonemia despite the presence of hyperglycemia or euglycemia. On the contrary, there are hepatic glycogen-storage disorders such as glycogenoses (absence of glucose-6-phosphatase) in which normal basal plasma glucagon level is associated with fasting hypoglycemia (23). These observations have led Kabadi (11, 12) to suggest that the hyperglucagonemic states are characterized by a unique metabolic environment, namely hepatic glycogen depletion.

Similar to the response in resting conditions, plasma glucagon response during prolonged exercise is often considered as a counterregulatory response to a decreasing glycemia. This view is supported by the demonstration that the rise in glucagon is abolished when blood glucose is increased by glucose ingestion before or during exercise (29). However, it is possible to find exercise studies where this feedback mechanism appears to break down. For instance, the glucagon response during prolonged exercise in dogs (1) and in humans (9) is not affected by a glucose infusion equivalent to the normal hepatic glucose production. In a recent study (2), reduced circulating fat in dogs resulted in a 50% greater glucagon levels during exercise without any systematic differences in plasma glucose. The purpose of the present investigation was, therefore, to test the relationship between the glucagon response and some variations in plasma glucose concentration during a period of prolonged exercise. Our a priori hypothesis is that blood glucose concentration is not always related to glucagon response during exercise.

METHODS

Animal care. Male Sprague-Dawley strain rats (Charles River Canada, St-Constant, PQ), weighing 230–250 g, were housed individually and fed pelleted rat chow and tap water ad libitum for 10 days after they were received in our laboratory. The 12:12-h light-dark cycle started at 7 AM, and the room temperature was maintained between 20 and 23°C. All rats were subjected to a habituation swimming protocol held on 3 consecutive days for 1, 1.5, and 2 h, respectively. All rats were gaining weight before inclusion in the study.

Surgery. Five days before experimentation, all rats underwent a right jugular vein and a left carotid artery cannulation under pentobarbital sodium (40 mg/kg i.p.) anesthesia. After insertion, the catheters were filled with saline containing heparin (500 U/ml; Fisher Scientific) and the external portion was capped with the crunched shaft of a blunted 23-gauge needle. Subsequently, 5 days were allocated for recovery.
Group and exercise protocol. The day before experimentation, rats were divided into three groups: two food-restricted groups and one normally fed group. The food-restricted rats received only 50% (10 g) of their daily food intake the night before experimentation. During the exercise protocol, one group of food-restricted rats was infused with glucose to maintain glycemia while the other groups received an equivalent infusion of isotonic saline. The exercise protocol consisted of a 3-h swim in a 30 x 41-cm tank filled to a depth of 76 cm with water maintained between 35 and 37°C and agitated by using compressed air. The pool was divided into two sections with a piece of Plexiglas, allowing the evaluation of two rats at the same time.

On the day of the experiment, food was removed from cages of fed rats at 7:00 AM, and the exercise tests were run between 8:00 AM and 1:00 PM. The catheters of the jugular vein and the carotid artery were connected to tubing extensions to be used for glucose infusion and blood sampling, respectively. After this procedure, rats were returned to their cages for a 40-min stabilization period. During the experimental protocol, blood was collected via the arterial catheter at rest (−20 and 0 min) and at different time intervals during the next 3 h. Collected blood (between 0.075 and 2.0 ml) was simultaneously replaced with whole blood from an anesthetized donor animal submitted to the same nutritional conditions as the experimental animal. The intravenous infusion of either glucose (25% dextrose solution, mean infusion rate: 1.75 µl/min) or isotonic saline (0.9%) was made by using a microinfusion pump (model 55-2222, Harvard Apparatus). Plasma glucose concentrations were measured every 20 min during the first 2 h and every 10 min during the last hour, and the infusion rate was adjusted to maintain plasma glucose concentration >120 mg/dl throughout the exercise session. At the end of the exercise period, the animals were rapidly taken out of the water and quickly anesthetized through the arterial catheter by using pentobarbital sodium (20 mg/kg). Immediately thereafter, the abdominal cavity was opened and a small piece of liver from the left lobe was frozen with aluminum block tongs cooled to liquid nitrogen temperature.

Analytic methods. Arterial blood was collected into heparinized syringes and separated into three fractions. The first aliquot of blood (500 µl) was preserved in Trasylol (50 µl) and centrifuged for 5 min, and the plasma was stored for glucagon determination. The second fraction of blood was centrifuged for 5 min (1 min for glucose), and the supernatant was retained for glucose and insulin analyses. The remaining part of blood was used for catecholamine determinations; it was transferred to microtubes containing 50 µl of gluthathione (60 mg/ml) and ethylene glycol-bis(β-aminooethyl ether)-N,N,N',N'-tetraacetic acid (90 mg/ml), kept on crushed ice, and centrifuged immediately for 10 min (5°C; 3,500 revolutions/min). All tissues and blood plasma were stored at −80°C until analyses were performed.

Plasma glucose concentrations were determined with the use of a glucose analyzer (model 2300, Yellow Springs Instrument, Yellow Springs, OH). Insulin and glucagon levels were determined by commercially available radioimmunoassay kits using porcine insulin and human glucagon standards, respectively (ICN Biomedicals, Costa Mesa, CA; distributed by Immunocorp, Montréal, PQ). Catecholamines were extracted from the plasma according to the procedure described by Remie and Zaagsma (24) and determined by means of an isocratic high-performance liquid chromatography system (Waters Division, Millipore). The recovery of norepinephrine, epinephrine, and dihydroxybenzylamine with the concentrations of 2 ng/ml was 95.8 ± 8.4, 94.5 ± 4.6, and 79.1 ± 4.3%, respectively. Liver glycogen content was determined by use of the phenol-sulfuric acid reaction (18).

Statistical analyses. All data are reported as means ± SE. The total area under the concentration curve and above the baseline for glucose was calculated by using a trapezoidal model. The blood variables were analyzed by a two-way analysis of variance with repeated-measures design. The Tukey post hoc test was used in the event of a significant (P < 0.05) F-ratio. Comparisons of liver glycogen values and mean areas under the glucose curves were done by using a one-way analysis of variance.

RESULTS

The hepatic glucose concentrations measured only at the end of exercise were 0.77 ± 0.14, 0.3 ± 0.02, and 0.3 ± 0.02 g/100 g for the fed rats and the two food-restricted groups of rats, respectively. These values were significantly (P < 0.01) higher in the fed than in the food-restricted rats. Plasma glucose concentrations were significantly (P < 0.01) reduced at 75, 105, 150, and 170 min of exercise (from ~130 to 110 mg/dl) in the food-restricted rats without glucose infusion (Fig. 1). The mean total areas under the glucose-concentration curve for the fed and the food-restricted, with and without glucose infusion, groups were 30,650 ± 1,140 (SE), 26,139 ± 804, and 24,619 ± 795 mg·dl⁻¹·180 min⁻¹, respectively. A significant (P < 0.01) difference in these mean integrated areas was found only between the fed and the two food-restricted groups. A significant (P < 0.05) increase in exercising plasma glucose was observed in the glucose-infused rats. In the fed rats, plasma glucose concentrations increased significantly (P < 0.01) throughout the exercise period (Fig. 1).
Plasma insulin concentrations decreased significantly 
\( P < 0.01 \) and similarly in all groups during exercise (Fig. 2). Plasma epinephrine and norepinephrine concentrations increased significantly \( P < 0.01 \) with exercise in all three groups (Fig. 3). No significant intergroup differences were found for the catecholamine response to exercise. Glucagon concentrations were, overall, similarly increased \( P < 0.01 \) in all three groups (from \( \sim 250 \) to \( 550 \) pg/ml) at the end of the exercise period (Fig. 4). Glucagon concentrations started to increase significantly \( P < 0.05 \) after 90 min of exercise in the food-restricted groups of rats and only after 140 min in the fed groups of rats (Fig. 4).

**DISCUSSION**

It is the general view that the decline in plasma glucose concentrations is the major determinant of the increase in glucagon secretion during prolonged exercise. However, it is possible to find deviations from this general concept in earlier studies (for a review see Ref. 5) as well as in more recent investigations (1, 2, 9). The present study was designed to investigate the possibility that glucagon secretion during exercise is not always related to a decrease in plasma glucose concentration. One of the approaches chosen was to maintain glycemia in food-restricted rats. The results show that, compared with resting values, blood glucose concentrations were significantly \( P < 0.05 \) decreased at 75, 105, 150, and 170 min (Fig. 1) in fasted rats. No such decrease was found throughout exercise in the fasted group of rats infused with glucose or in normally fed rats. Despite these differences in plasma glucose levels, glucagon concentrations were increased significantly at the end of the exercise period in all three groups (Fig. 4). The increase in glucagon during exercise is even more puzzling if one considers that overall blood glucose levels were not decreased during exercise in the food-restricted rats without glucose infusion, as indicated by the areas under the curve. An important distinction in the glucagon response between the groups, however, is the observation that glucagon levels started to increase after 90 min of exercise in both fasted groups of rats, irrespective of the glucose infusion, whereas it started to increase only after 140 min of exercise in the fed group. Overall, these data indicate that an absence of a decrease in blood glucose level during a prolonged exercise period did not prevent

**Fig. 2.** Plasma insulin concentrations at rest and during exercise in fast and fed rats, with and without glucose infusion. Values are means \( \pm SE \) for 10–12 rats at each point. \( \square \), Fast + glucose infusion; \( \blacksquare \), fast; \( \bullet \), fed. *Significantly different from all corresponding exercise values, \( P < 0.05 \).

**Fig. 3.** Plasma epinephrine and norepinephrine concentrations at rest and during exercise in fast and fed rats, with and without glucose infusion. Values are means \( \pm SE \) for 10–12 rats at each point. \( \square \), Fast + glucose infusion; \( \blacksquare \), fast; \( \bullet \), fed. *Significantly different from corresponding resting values, \( P < 0.05 \).
glucagon response to be largely increased. In addition, it seems whether the rats were in the fed or fasted state before exercise influences the time sequence of the subsequent glucagon response. These data, therefore, represent a good indication that a decrease in blood glucose level cannot be the sole determinant of glucagon secretion during exercise.

Similar to the glucagon response, epinephrine and norepinephrine levels were increased in all three groups during exercise. Because these catecholamines were measured only at two time points during exercise, it cannot be known if the time course of these responses was the same for the three groups. There is a tendency for the epinephrine response to be increased more rapidly in the fasted groups of rats without glucose infusion. The catecholamine responses deserve closer consideration because it has been suggested that, in rats, glucagon secretion is mainly due to sympathetic stimulation of alpha-cells (5). This concept, however, is far from being unanimous. For instance, glucose administration in rats has been reported to reduce the exercise-increased glucagon concentrations in some studies (8, 19). It has been reported (5) that the time course of glucagon secretion in exercising rats is similar to that of catecholamine responses. A close look at the data, however, shows that the same relationship of plasma glucagon response could also be established with blood glucose or liver glycogen (30). Contradictory data have led some authors to suggest that both plasma glucose and plasma catecholamines play a major role in exercise-induced glucagon secretion in rats (6). It has also been suggested that in rats unidentified factors are of importance for the glucagon response to exercise (6, 25). In the present study, it cannot be excluded that plasma catecholamines may be associated with the similar increase in glucagon levels measured at the end of exercise. However, both epinephrine and norepinephrine concentrations were significantly elevated after 120 min of exercise in fed rats, which was not the case for glucagon concentrations. It is thus possible that in addition to glucose and/or catecholamines, another factor might be of importance for the glucagon response to exercise.

The reason for using fasted and fed rats in the present study was to try to draw a parallel between the level of hepatic glycogen content and the glucagon response during exercise. The results of our study show that hepatic glycogen values were low in all groups at the end of exercise, representing at most 10% of normal values in fed conditions (16). This might constitute a possible explanation for the similar increase in glucagon in all three groups at the end of exercise. In addition, the time course of glucagon increases during exercise was lower in the fed group of rats than in both groups of fasted rats (Fig. 4). It is possible that hyperglycemia in the fed rats might have acted to prolong the time before the increase in glucagon. However, this would not explain why glucagon in the same group of rats was sharply increased between 140 and 180 min of exercise while hyperglycemia continued to increase. Although we do not have a time course of the decrease of liver glycogen during exercise, it is reasonable to assume that liver glycogen concentrations were higher in the fed group than in the fasted groups throughout exercise. It is, alternatively, possible that the slower glucagon response during exercise in the fed rats may be associated with a higher liver glycogen content. Throughout the literature, several physiological and pathological states have been shown to have a distinct relationship between hepatic glycogen content and plasma glucagon level (for a review, see Ref. 11). Although the nature of the feedback regulatory mechanism between the liver and the pancreatic α-cell is not known, it has been hypothesized that the hepatic cells secrete a hormonal factor, as a result of high level of hepatic glycogen content, which would suppress glucagon secretion (11). Conversely, if hepatic glycogen stores are depleted, the secretion of this substance would be inhibited, resulting in hyperglucagonemia. In the absence of a clear relationship between plasma glucose and glucagon response, it is possible that this overall mechanism might contribute to the increase in glucagon secretion during a period of prolonged exercise. It is suggested that a new concept relating liver glycogen content to glucagon might be of importance for the glucagon response to exercise.

The infusion of glucose during exercise did not have any effect on plasma insulin response compared with the noninfused groups. This is an indication that the quantity of glucose infused was modest. In addition, the low level of liver glycogen at the end of exercise in the infused rats suggests that the glucose infusion did not prevent the remaining liver glycogen from being used during exercise. On the other hand, the increase in blood glucose levels in the fed rats during exercise needs to be addressed. These increases started at the
very beginning of the exercise period (20 min) and might reflect a stress component of the swimming exercise. However, because the plasma catecholamine response was not higher in the fed group than in the other groups it cannot be why the glucagon response started to increase only after 140 min of exercise.

In summary, the present study provides evidence that a decrease in blood glucose concentration cannot be the sole determinant of the glucagon response during exercise. It is suggested that a new concept relating liver glycogen content to glucagon might be of importance for the glucagon response to exercise.

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