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

FRANÇOIS DÉSY, CLAUDE WARREN, AND JEAN-MARC LAVOIE


Abstract

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. 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 habitation 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 ip) 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 experimenta-
tion, 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 equiva-
 lent infusion of isotonic saline. The exercise protocol consisted
of a 3-h swim in a 30 × 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 exten-
sions 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 experi-
mental 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 anesthe-
tized donor animal submitted to the same nutritional condi-
tions 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 maintained at
a constant rate 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
aluminium block tongs cooled to liquid nitrogen temperature.

Analytic methods. Arterial blood was collected into heparin-
ized 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 gluca-
gon 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 glutathione (60
mg/ml) and ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-
tetraacetic acid (90 mg/ml), kept on crushed ice, and centri-
fuged 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 Instru-
ment, 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 Immunoncorp, 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 concentra-
tion 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 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 concentra-
tions 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...
glucagon response to be largely increased. In addition, it seems that 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 a-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.

We thank Nathalie Rhéaume for excellent technical assistance. This work was supported by Natural Sciences and Engineering Research Council of Canada and Fonds pour la Formation des Chercheurs et l’Aide à la Recherche (Government of Quebec).

Address for reprint requests: J.-M. Lavoie, Département d’Éducation Physique, Université de Montréal, C.P. 6128, succ Centre-ville, Montréal, PQ, Canada H3C 3J7 (E-mail: lavoije@ere.umontreal.ca).

Received 31 December 1996; accepted in final form 17 March 1997.

REFERENCES

