Influence of prior exercise and liver glycogen content on the sensitivity of the liver to glucagon

VICTORIA MATAS BONJORN, MARTIN G. LATOUR, PATRICE BÉLANGER, AND JEAN-MARC LAVOIE
Département de Kinésiologie, Université de Montréal, Montreal, Quebec, Canada H3C 3J7

Received 25 May 2001; accepted in final form 5 September 2001

Bonjorn, Victoria Matas, Martin G. Latour, Patrice Bélanger, and Jean-Marc Lavoie. Influence of prior exercise and liver glycogen content on the sensitivity of the liver to glucagon. J Appl Physiol 92: 188–194, 2002.—The purpose of the present study was to test the hypothesis that a prior period of exercise is associated with an increase in hepatic glucagon sensitivity. Hepatic glucose production (HGP) was measured in four groups of anesthetized rats infused with glucagon (2 μg·kg−1·min−1 iv) over a period of 60 min. Among these groups, two were normally fed and, therefore, had a normal level of liver glycogen (NG). One of these two groups was killed at rest (NG-Re) and the other after a period of exercise (NG-Ex; 60 min of running, 15–26 m/min, 0% grade). The two other groups of rats had a high hepatic glycogen level (HG), which had been increased by a fast-refed diet, and were also killed either at rest (HG-Re) or after exercise (HG-Ex). Plasma glucagon and insulin levels were increased similarly in all four conditions. Glucagon-induced hyperglycemia was higher (P < 0.01) in the HG-Re group than in all other groups. HGP in the HG-Re group was not, however, on the whole more elevated than in the NG-Re group. Exercised rats (NG-Ex and HG-Ex) had higher hyperglycemia, HGP, and glucose utilization than rested rats in the first 10 min of the glucagon infusion. HG-Ex group had the highest HGP throughout the 60-min experiment. It is concluded that hyperglucagonemia-induced HGP is stimulated by a prior period of exercise, suggesting an increased sensitivity of the liver to glucagon during exercise.

HEPATIC GLUCOSE PRODUCTION (HGP) is a metabolic process that is absolutely essential for the maintenance of glycemia in postprandial periods as well as during exercise (21, 23). To maintain adequate blood glucose supply, hepatic glycogenolysis and gluconeogenesis processes are regulated by complex interactions of metabolic and hormonal stimuli (13). Among these stimuli, glucagon secretion in relation to exercise has been the subject of considerable interest over the years (for a review, see Refs. 25 and 27). From these studies, it appears that the existence of basal glucagon levels is clearly required at rest and during exercise to attain typical glucose production rates (13, 26). Furthermore, the role of glucagon as a primary controller of hepatic glycogenolysis and gluconeogenesis during exercise has been recognized (28).

Although the contribution of glucagon to the increment in HGP is well recognized, very few studies have looked at how the liver modulates the action of glucagon during exercise. For instance, there is one report that higher than normal initial levels of liver glycogen than increase HGP during exercise (24). More recently, there has been one report that livers of trained individuals are more responsive to a glucagon infusion than untrained individuals (10). The authors mentioned that trained individuals are most likely to have higher resting levels of liver glycogen as well as higher levels of muscle glycogen (1, 11). Subsequently, the same group of authors reported an increased density of glucagon receptors from endurance-trained rats (15). This finding strongly supports the view that chronic exercise increases the sensitivity of the liver to glucagon. It is not known, however, if an acute bout of exercise may also increase the sensitivity of liver cells to glucagon. Such a phenomenon has been observed with insulin, in which the sensitivity of muscle cells to insulin has been reported to be increased after chronic (18) as well as after acute bouts of exercise (5).

The purpose of the present study was to determine if an acute bout of exercise influences the extent to which HGP and related variables (plasma glucose and insulin levels) are changed by a constant 60-min infusion of glucagon. It was hypothesized that a prior bout of exercise would result in an increased sensitivity of the liver to glucagon. The possibility that a prior period of exercise could affect hepatic sensitivity to glucagon was examined in normal glycogen (NG) and supranormal (HG) liver glycogen conditions.

METHODS

Animal care. Male Sprague-Dawley rats (Charles River, St. Constant, Canada), weighing 220–240 g, were housed in individual cages and allowed pelleted rat chow and tap water ad libitum for −13 days after they were received in our laboratory. The lighting schedule was such that lights were on from 0700 until 1900, and room temperature was main-
tained at 20–23°C. Two days after their arrival, rats underwent a habituation running protocol on a motor-driven rodent treadmill consisting of six sessions over an 8-day period, beginning with 20 min/day at 15 m/min and progressively increased to 60 min/day at 26 m/min (0% grade), so that they were well accustomed to running and being handled. The last habituation session was held 2 or 3 days before the experiment for all rats.

Groups and surgery. After completion of their running habituation protocol, rats were randomly assigned to one of the four experimental groups. One group of rats was normally fed and, therefore, had NG in the resting state (NG-Re). Another group of rats had a high level of liver glycogen in the resting state (HG-Re). This was done by submitting the rats to a 24-h fast before access to the normal Chow diet. The third group of rats was normally fed but was subjected to 60 min of running exercise on a treadmill (NG-Ex; running for 10 min at 15 m/min, 20 min at 20 m/min, and 30 min at 26 m/min, 0% grade) before the beginning of the experiment. The fourth group of rats underwent both the fast-refed and exercise protocols (HG-Ex) described above.

The morning of the experiment, rats in NG-Ex and HG-Ex groups were first submitted to the 1-h exercise bout described above by a 15-min rest period before being submitted to the same surgical and experimental manipulations as rats in the other groups. The morning of the experiment, rats in all groups were weighed and anesthetized with pentobarbital sodium (40 mg/kg ip) as the whole experiment was conducted by an independent researcher using curve fitting by hand (2).

Analytical methods. Blood was collected into 1-ml syringes with 7% EDTA. For the assay of [3-3H]glucose radioactivity, 70 μl of plasma were deproteinized with 35 μl of 1 N perchloric acid and centrifuged (14). Duplicate aliquots (30 μl) of the supernatant were evaporated overnight under a stream of air to remove tritiated water. The dry residue was redissolved in 200 μl of water and counted in 3 ml scintillation liquid (Sigma-Fluor, Sigma Chemical, St. Louis, MO) in a liquid scintillation spectrophotometer. Correction for counting efficiency was always carried out by means of dilutions of the infusate with plasma run in parallel with plasma samples (14). The fraction of blood (150 μl) to be used for glucagon determination was preserved in Trasylol (50 μl) before centrifugation. The remainder of the blood was also centrifuged (Eppendorf centrifuge, no. 5415), and the plasma was stored for subsequent glucose and insulin determinations. All tissue and blood samples were stored at −78°C until analyses were performed.

Statistical analyses. All data are reported as means ± SE. The total area under the concentration curve and above baseline for glucose, glucose production, glucose utilization, insulin, and glucagon was calculated by using a trapezoidal model. Intergroup comparisons were conducted by using these areas calculated for the first 10 min (short-term response) and for the whole 60-min period of glucagon infusion. Statistical comparisons were completed by using two- and one-way ANOVA for repeated and nonrepeated measures design, respectively. A 95% level of confidence was accepted for all comparisons.

J Appl Physiol • VOL 92 • JANUARY 2002 • www.jap.org
RESULTS

Dietary and exercise manipulations resulted in pre-infusion liver glycogen concentrations that were all significantly different ($P < 0.01$) from one group to another (Fig. 1A). Liver glycogen content in the HG-Re group was approximately twice as much as liver glycogen content in the NG-Re group. After glucagon infusion, all groups had their liver glycogen content significantly decreased ($P < 0.01$; Fig. 1A). As presented in Fig. 1B, the extent of liver glycogen breakdown was the same in all groups with the exception of a slightly but significantly larger ($P < 0.05$) glycogen breakdown in the NG-Re than in the NG-Ex group.

Blood glucose concentrations before and in response to the glucagon infusion are presented in Fig. 2A. There were no significant intergroup differences in plasma glucose concentrations in the preinfusion period. Plasma glucose concentrations were significantly increased ($P < 0.01$) in all groups after glucagon infusion (Fig. 2A). The area under the glucose concentration curves during the first 10 min after the beginning of the infusion was significantly higher ($P < 0.01$) in the NG-Ex group compared with all other groups (Fig. 2B). For the whole 60-min period, the area under the glucose concentration curves was significantly higher ($P < 0.05$) in the HG-Re compared with all other groups, whereas the response of the HG-Ex group was significantly higher ($P < 0.05$) than that of the NG-Ex group (Fig. 2C).

HGP before and in response to glucagon infusion is presented in Fig. 3A. In the preinfusion period, there were no significant differences ($P > 0.05$) in glucose production between all groups. The infusion of glucagon resulted in a rapid increase ($P < 0.01$) in HGP in all groups. During the first 10 min of glucagon infusion, the area under the glucose production curves was significantly higher ($P < 0.01$) in the NG-Ex group than in all other groups with the exception of the HG-Ex group ($P < 0.06$; Fig. 3B). During the same time, the response of the HG-Ex group was significantly higher ($P < 0.05$) than the response in the HG-Re group (Fig. 3B). For the whole 60-min period, the area under the glucose production curves was significantly greater ($P < 0.05$) in the HG-Ex group compared with all groups with the exception of the HG-Re group ($P < 0.08$; Fig. 3C).

Glucose utilization before and in response to the glucagon infusion is presented in Fig. 4A. In the preinfusion period, glucose utilization was similar in all groups. Glucose utilization was significantly ($P < 0.01$)
increased in all groups after glucagon infusion. During the first 10 min of glucagon infusion, the area under the glucose utilization curves was significantly higher (P < 0.05) in the NG-Ex group than in all other groups with the exception of the NG-Re group (Fig. 4B). For the whole 60-min period, the total area under the glucose utilization curves was significantly higher (P < 0.05) in the HG-Ex than in the HG-Re group (Fig. 4C).

As expected, plasma glucagon concentrations were significantly increased (P < 0.01) after glucagon infusion (Fig. 5A). There were no significant intergroup differences (P > 0.05) in the glucagon concentrations at any of the experimental times. Plasma insulin concentrations were also increased significantly (P < 0.01) after glucagon infusion (Fig. 5B). No intergroup differences (P > 0.05) in insulin concentrations were found at the different time points during the first 45 min of glucagon infusion and for the overall 60-min period (total area). Glucagon-insulin molar ratio (data not shown) increased from values of ~0.2 in the preinfusion period to values ~100-fold larger during infusion.

No significant intergroup differences were found at any times in the glucagon-insulin molar ratio. Glycogen concentrations from the soleus muscle, measured only at the end of the experiment, were higher (P < 0.05) in the HG-Re group than in all other groups with the exception of the HG-Ex group (Fig. 5C).

DISCUSSION

The purpose of the present experiment was to determine the impact of a prior period of exercise on the sensitivity of the liver to glucagon. Data of the present study, conducted under normal and supranormal initial levels of liver glycogen, are the first to indicate that exercise has sensitized the liver to the action of glucagon.

Exercise effect with normal liver glycogen content.

The first observation from the present data is that, in the first 10 min of glucagon infusion, prior exercise in the NG-Ex group resulted in a larger increase in glucose production than the one measured in the NG-Re group.
group. This increase in glucose production during the first 10 min of glucagon infusion was ~30 to 40% higher in the NG-Ex than in the two resting groups (NG-Re and HG-Re) despite lower initial levels of liver glycogen in the NG-Ex group. Dobbins et al. (9) have recently reported data showing that a step increase in glucagon results in rapid stimulation of glucose production reaching half-maximal stimulation in just 4.5 min. The early larger increase in glucose production in the NG-Ex group resulted in a concomitantly larger increase in plasma glucose concentration in the first 10 min, even though glucose utilization was also much more increased in that group than in most other groups during the same time (Fig. 4B). The observation of a larger increase in glucose utilization in the NG-Ex group was expected because acute exercise is well known to stimulate glucose utilization, most likely at the muscular level (5). The present results in normally fed rats are, however, the first to demonstrate that acute exercise also increases hepatic glucagon sensitivity.

The early increase in glucose production and utilization in the NG-Ex group was not, however, carried on during the remainder of the experiment as glucose production and utilization, as well as plasma glucose values, were steadily decreased during the last 40 to 50 min of the experiment. This is most likely due to reduced initial levels of liver glycogen in the NG-Ex group and could explain why the larger glucagon-induced increase in HGP in the NG-Ex group was observed only in the first 10 min of glucagon infusion.

Exercise effects with supranormal liver glycogen content. As mentioned above, one of the drawbacks of studying the effects of a prior period of exercise on glucagon-induced HGP is the fact that, after exercise, liver glycogen content is decreased. We tried to circumcribe this problem in the present study by examining the effects of a prior period of exercise on glucagon-induced HGP in a condition in which liver glycogen level was still above normal levels after exercise, as it was in the HG-Ex group. The first observation from the present data using this approach is that, in the first 10 min of glucagon infusion, glucose production was significantly larger in the HG-Ex group than in the HG-Re group (Fig. 3B). This confirms our previous observation, in normally fed rats, that prior exercise rapidly increases the HGP response to gluca-
Excrete subsequent glucagon-induced liver glycogen breakdown (Fig. 1B). It may be argued that initial liver glycogen levels were reduced in the NG-Ex group compared with the NG-Re group. However, glucagon-induced liver glycogen breakdown after exercise was the same in the NG-Ex group compared with the HG-Ex group, whereas initial levels of liver glycogen were twice as much in the HG-Ex group than in the HG-Ex group. Overall, within the limits of the measurement of liver glycogen content over a 60-min period, it appears that a supranormal initial level of liver glycogen or a prior period of exercise do not increase liver glycogen breakdown. This, however, does not invalidate the fact that glucagon-induced HGP was different among the present groups, because measurements of liver glycogen decreases do not take into account glucose produced by the gluconeogenesis pathway or glycogen that may have been resynthesized during the course of glucagon infusion.

There are limits to the interpretation of the present data that need to be addressed. Plasma glucagon concentrations in the present experiment were increased similarly in all groups, indicating that the different HGP responses among groups were not due to different glucagon concentrations. The interpretation of the present data is, however, limited to the situation of hyperglucagonemia. There is a possibility that the observed effects might have been different with a more physiological infusion of glucagon. On the other hand, exogenous hyperglucagonemia, by saturating the receptors, eliminates the possibility that the action of the hormone is limited by its secretion or by its plasma concentration. It is also possible that the glucagon-induced reactive hyperinsulinemia might have interfered differently among groups with the effects of glucagon. There was, however, no significant differences in insulin responses among groups. In vivo and in vitro studies have also indicated that the effects of insulin in liver may be overridden by high doses of glucagon (4, 17), as was the case in the present study. Finally, the present data do not provide any clear information as to what extent gluconeogenesis and/or glycogenolysis have been respectively stimulated by glucagon infusion. It is interesting to observe that the groups (NG-Re and NG-Ex) showing the lowest values for glucose production and plasma glucose values during the last 30 min of glucagon infusion also show the lowest initial and final levels of liver glycogen. This suggests, as previously reported (7), that the present glucagon-induced glucose production depends to a large extent on hepatic glycogenolysis, although it is not excluded that gluconeogenesis was also stimulated.

In summary, results of the present experiment indicate that hyperglucagonemia-induced HGP is increased in a postexercise situation. It is suggested that, in addition to the increase in glucagon secretion, HGP during exercise is also stimulated by an increased sensitivity of the liver to glucagon.
REFERENCES


