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

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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 (HG) liver glycogen conditions. 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 than rested rats in 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 pellet 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-

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. To maintain adequate blood glucose supply, hepatic glycogenolysis and gluconeogenesis processes are regulated by complex interactions of metabolic and hormonal stimuli. Among these stimuli, glucagon secretion in relation to exercise has been the subject of considerable interest over the years. The role of glucagon as a primary controller of hepatic glycogenolysis and gluconeogenesis during exercise has been recognized.

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).

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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 followed 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 performed with the animals under anesthesia. All rats were then implanted with two catheters into the right jugular vein for the respective infusions of glucagon and [3-3H]glucose, and one into the carotid artery for blood sampling. All catheters were filled with sterile 0.9% NaCl. A small midabdominal incision (2–2.5 cm) was thereafter made to give free access to the liver. An open liver biopsy (−100 mg) was then taken according to Daemen et al. (8). After the liver was presented via the abdominal incision, a small piece of liver tissue (80–100 mg) was cut out of the edge of a liver lobe with a sharp scissors cooled to liquid-nitrogen temperature. The use of a cooled scissors allowed almost no bleeding. After the biopsy, the abdomen was closed. The whole surgery period lasted ~15 min and was followed by a 10-min recovery period before the beginning of the infusions. The body temperature of the animals during the entire experiment was maintained by using a heating blanket.

Experimental protocol. On the morning of the experiment, any remaining food was removed from the cages 3 h before the experiment, which was conducted between 0700 and 1300. After the exercise (when appropriate) and surgical procedures described above, a 75-min period was allowed to anesthetized rats for tracer stabilization ([3-3H]glucose) with arterial blood samples taken every 5 min during the last 10 min of this period for the determination of the basal rate of glucose turnover. This period was immediately followed by a 60-min period of glucagon infusion in all rats. For this period, a primed (20 μg/kg over 1 min) constant-rate (2 μg·kg⁻¹·min⁻¹) infusion of glucagon (from porcine pancreas; Sigma-Aldrich Canada, Oakville, Canada) dissolved (20 μg·kg⁻¹·ml⁻¹) in diluting fluid (1.6% glycerine and 0.2% phenol) with saline (12) was delivered intravenously by a precision pump (Harvard Apparatus). This dose of glucagon was chosen to mimic the bolus dose recently used by Rao (20) to demonstrate that chronically malnourished rats are glucagon resistant in the liver. During the infusion period, minor arterial blood samples (250 μl) were taken every 5 min for determination of glucose turnover and major arterial blood samples (1 ml) were taken every 15 min for additional measurements of insulin and glucagon. Red blood cells obtained from blood centrifuged without preservatives were resuspended in saline and reinjected into the animal. After the last blood sample was taken, the abdominal cavity was reopened and a piece of liver was removed and frozen with aluminum block tongs cooled to liquid nitrogen temperature. Immediately afterward, the soleus muscle of the right leg was removed, freeze clamped, and placed in liquid nitrogen.

Glucose turnover. A primed (74 kBq) continuous infusion of [3-3H]glucose (dissolved in ultra-pure water, 1.480 kBq/ml, >97% pure; New England Nuclear, Boston, MA) was delivered intravenously at a precision pump (Harvard Apparatus). The infusion rate of the tracer during the stabilization period was 2.457 kBq/min (~75 to 0 min). This rate was increased twofold to 4.914 kBq/min at the beginning of the glucagon infusion and continued during the glucagon infusion period. This was done to keep the tracer-specific activity as constant as possible. Glucose appearance and disappearance were calculated by using the formula for nonsteady-state conditions developed by Steele (22) and validated by Radziuk et al. (19). The volume of distribution in which rapid changes in glucose concentration and specific activity of [3-3H]glucose take place was set to 188 ml/kg body wt (6). Smoothing of glucose and specific activity values to reduce rapid changing of these variables with respect to time 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-Flur, Sigma Chemical, St. Louis, MO) in a liquid scintillation spectrophotometer. Correction for counting efficiency was always carried out by means of dilutions of the infused 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.

Plasma glucose concentrations were determined by using a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Insulin and glucagon concentrations were determined by commercially available radioimmunoassay kits (Radioassay System Laboratory, ICN Biomedicals, Costa Mesa, CA; distributed by Immunocorp, Montreal, Quebec, Canada). Liver and muscle glycogen concentrations were determined by using phenolsulfuric acid reaction (16). 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.
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 \( (P < 0.01; \text{Fig. 1A}) \). 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. 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 \( (P < 0.06; \text{Fig. 3B}) \). 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 \( (P < 0.08; \text{Fig. 3C}) \).

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 \(\sim 0.2\) in the preinfusion period to values \(\sim 100\)-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 (Fig. 4).
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 circumvent this problem in the present study by examining the effects of a prior period of exercise on glucagon-induced HGP 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-
In addition, in the HG-Ex condition, overall glucose production (60 min) was maintained to the highest levels compared with all other groups (P < 0.08 vs. the HG-Re group, even though preinfusion liver glycogen content was higher in the HG-Re group than in the HG-Ex group; Fig. 3C). This indicates that the stimulating effect of a prior period of exercise on HGP response to glucagon remains active for a long period of time (60 min), providing liver glycogen is present in a sufficient amount. The fact that glucagon-induced glucose production was different between the two exercising groups (HG-Ex > NG-Ex) ruled out the possibility that the present effects of exercise on HGP may be simply due to an increased availability of gluconeogenic precursors. On the other hand, although glucose production for the whole 60-min period was larger in the HG-Ex group, plasma glucose concentrations were not maintained as high in that group as they were in the HG-Re group (Fig. 2C). This may be simply explained by the fact that muscle glucose utilization is well known to be stimulated in the postexercise situation. Nevertheless, one might argue that the higher levels of HGP in HG-Ex rats compared with HG-Re rats for the whole 60-min period is due to lower plasma glucose concentrations found in the HG-Ex group vs. the HG-Re group. One has to remember, however, that all plasma glucose values during glucagon infusion were in the hyperglycemic range in both groups, therefore, contributing to the inhibition of HGP in both groups. In addition, similar differences between levels of hyperglycemia in rats in HG-Ex groups compared with NG-Re groups resulted in no difference in HGP between these two groups. Overall, data in the supernormal liver glycogen condition confirm what has been observed in the normally fed state, which is that prior exercise is associated with an early (10 min) larger hyperglucagonemia-induced glucose production, which can be carried on for a longer period of time (60 min) providing that enough liver glycogen is available. These data strongly support the above-mentioned suggestion that liver sensitivity to glucagon is increased by an acute bout of exercise as it is increased in response to chronic exercise training (10). It can be postulated that the density of glucagon receptors in the liver can be rapidly increased by an acute bout of exercise as it has recently been shown to increase after an endurance training program (15).

Liver glycogen breakdown. Liver biopsies were taken at the beginning and at the end of the present experiment to get some information on glucagon-induced liver glycogen breakdown. This measurement, at first glance, indicates that overloading the liver in glycogen does not increase glucagon-induced liver glycogen breakdown. This is in agreement with what we recently reported for a 60-min period following glucagon injections in similarly overloaded rats (3). In the latter study (3), however, hyperglucagonemia-induced liver glycogen breakdown was accentuated in the liver glycogen-overloaded group in the subsequent 30 min (minutes 60–90). Results of the present study also indicate that a prior period of exercise does not increase 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 NG-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.

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