Evidence That a Decrease in Liver Glycogen Content Stimulates FFA Mobilization During Exercise

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Catalog Data

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Abstract/Résumé
This study evaluated a liver glycogen content decrease before exercise on the metabolic responses during exercise. Rats injected with glucagon (20 μg · kg⁻¹) were compared to rats with a 50% food restriction (1/2-fast) and normally fed rats. All were studied at rest and during exercise (26 m/min, 0% grade). Resting liver glycogen concentrations were twice as high (P < .01) in normally fed rats, with no significant differences between 1/2-fast and glucagon-injected rats. During exercise, liver glycogen content was significantly reduced in normally fed rats. After exercise, plasma insulin levels were decreased (P < .01) in all groups, and β-hydroxybutyrate concentrations were similar in normally fed and glucagon-injected rats and significantly (P < .01) lower in 1/2-fast rats. Exercise caused a significant increase in FFA concentrations in all groups (P < .01). No significant differences in FFA concentrations were found between 1/2-fast and glucagon-injected groups (P > 0.05).

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Le but de la présente étude était d’évaluer les effets d’une diminution pré-exercice du contenu de glycogène hépatique, obtenue sans une réduction de la quantité de nourriture ingérée, sur les réponses métaboliques de l’exercice. Les niveaux de glycogène hépatique pré-exercice furent réduits par trois injections de glucagon (20 µg · kg⁻¹ · aux 20 min) dans un groupe de rats, lesquels furent comparés à un groupe de rats soumis à une restriction alimentaire de 50% (1/2-jeûne) la veille de l’expérience et à un 3ème groupe de rats normalement nourris. Tous les groupes furent, par après, étudiés au repos et au cours de périodes d’exercice de 30 et de 50 min (26 m/min, 0% pente). Les concentrations de glycogène hépatique au repos étaient 2 fois plus élevées (P < .01) chez les rats normalement nourris comparativement aux animaux des deux autres groupes, alors qu’aucune différence significative ne fut observée entre ces deux derniers. Au cours de l’exercice, le contenu en glycogène hépatique diminuait seulement chez les rats normalement nourris. Les concentrations plasmatiques de glucose sanguin furent maintenus tout au long de l’exercice pour les 3 groupes. Les niveaux plasmatiques d’insuline diminuèrent (P < .01) après 50 min d’exercice pour les 3 groupes. À la fin de la période de 50 min d’exercice, les niveaux plasmatiques de β-hydroxybutyrate étaient similaires chez les groupes normalement nourris et injectés au glucagon, les valeurs de ces deux groupes étant significativement (P < .01) inférieures aux valeurs correspondantes mesurées chez les rats 1/2-jeûne. L’exercice provoqua une augmentation significative (P < .01) des niveaux plasmatiques d’AGL pour tous les groupes. Cependant, cette augmentation était plus marquée chez les rats injectés au glucagon (1.13 ± 0.09 mmol • L⁻¹; P < 0.01) et les rats 1/2-jeûne (1.18 ± 0.14 mmol • L⁻¹; P < .08) en comparaison des rats normalement nourris (0.8 ± 0.08 mmol • L⁻¹). Aucune différence significative dans les niveaux plasmatiques d’AGL à l’exercice ne fut observée entre les rats 1/2-jeûne et les rats injectés au glucagon (P > 0.05). Ces résultats indiquent qu’une réduction du contenu de glycogène hépatique est associée à une stimulation de la mobilisation des lipides au cours de l’exercice et cela, indépendamment des autres perturbations métaboliques généralement associées avec un état de restriction alimentaire ou d’apports alimentaires faibles en glucides.

Introduction

In recent years, evidence has been gathered showing that the liver may act as an afferent organ contributing to the metabolic and hormonal regulation of exercise (for a review, see Lavoie and Cardin, 1996). The best evidence in favor of such a role by the liver during exercise comes from the demonstration that hepatic vagotomy attenuates the exercise-induced reduction in insulin and increase in glucagon in adrenomedullated rats (Lavoie et al., 1989). The concept behind these observations is that the information about a decreasing liver glycogen content or glucose intermediates during exercise is carried on to the central nervous system or other organs contributing to the orchestrated metabolic response normally seen during exercise (Cardin et al., 1994). Evidence that the accessibility of intracellular substrates has been manipulated by changing total body carbohydrate stores through diets rich or poor in carbohydrates (Galbo et al., 1979; Jansson et al., 1982). More specifically, it has been suggested that a reduction in liver glycogen stores independent of a reduction in blood glucose levels may provide a causal stimulus to the metabolic and hormonal adaptations of prolonged exercise (Hélie et al., 1985; Lavoie et al., 1988). In these previous studies
(Galbo et al., 1979; Hélie et al., 1985; Jansson et al., 1982), as well as in more recent ones (Weltan et al., 1998a, 1998b), however, a reduction in liver and/or muscle glycogen content cannot be totally differentiated from a general state of reduction in carbohydrate, even though subjects were provided with an exogenous source of glucose. It can still be argued that a state of fasting or reduction in food intake can induce other metabolic adaptations, such as resting hypoinsulinemia (Galbo et al., 1981) or a change in internal milieu (Bray, 1997), that can influence the metabolic adaptations of exercise.

The aim of the present study, therefore, was to investigate the effects of a reduction in liver glycogen content, obtained without any restriction in food intake, on the metabolic responses to exercise. This was conducted by reducing liver glycogen content prior to exercise by injecting rats with glucagon and comparing the metabolic responses of subsequent exercise to a group of rats with similarly reduced liver glycogen content resulting from a food intake reduction and to a group of rats with a normal liver glycogen content.

Methods

ANIMAL CARE

Male Sprague-Dawley strain rats (Charles River, Canada, St. Constant, Quebec), weighing 240–260 g were housed in individual cages and allowed pellet rat chow and tap water ad libitum for 14 days after they were received in our laboratory. The lighting schedule was such that lights were on from 0600 until 1800, and the room temperature was maintained at 20–23°C. During this time, rats underwent a habituation running protocol on a motor-driven rodent treadmill consisting of ten sessions over two weeks, beginning with 15 min/day at 15 m/min and progressively increased to 55 min/day at 30 m/min (0% grade), so that they were well accustomed to running and being handled. Four days before the experimentation, all rats underwent a jugular vein cannulation under pentobarbital sodium (40 mg/kg ip) anesthesia. The jugular catheter was implanted by a method described previously (Lavoie et al., 1988). This catheter was used for rapid anesthesia (i.v.) of rats at the end of acute exercise or resting conditions.

GROUPS AND EXERCISE PROTOCOL

After the last training session, rats were randomly divided into three groups: 1/2 fasted group (1/2-fast), normally fed group, and normally fed group injected with glucagon immediately before the experiment (glucagon-injected). The 1/2-fast rats received only 50% of their daily food intake (~15 g) the night before the experimentation. Each group was further divided into three subgroups sacrificed at rest or after 30 or 50 min of exercise for a total of 9 groups. The exercise test consisted of running on the treadmill at 26 m/min (0% grade). On the morning of the experiment, food was removed from cages at 7:30 a.m., and the experiments were conducted between 9:30 a.m. and 12:30 p.m. Rats assigned to the glucagon-injected group received three bolus injections (at 0, 30, and 60 min; over a 1 min period) of glucagon (from porcine pancreas; Sigma-Aldrich Canada, Oakville) at a dose of 20 µg · kg⁻¹ dissolved in 1 mL diluting fluid (1.6% glycerine and 0.2% phenol)
with saline (Geary et al., 1993). This dose of glucagon has been shown to increase liver glycogen breakdown (Rao, 1995).

In one of our previous studies (Bélanger et al., 1998) using the same glucagon injection protocol, glucagon concentrations increased to ~3,000 pg · ml⁻¹, 15 min after the injection and decreased back to normal levels 30 min after the injection. Rats in the other groups were injected similarly with an equivalent volume of the vehicle only. The three injection protocol was followed by a 60-min rest period for all rats. Thereafter, rats were either run on the treadmill for 30 or 50 min or in the resting state in their cages for another 40 min. At the end of the resting or exercise period, rats were anesthetized via the venous catheter with pentobarbital sodium (20 mg · kg⁻¹) while still running or resting in their cages. Following complete anesthesia, the abdominal cavity was opened, and ~4 ml of peripheral blood were collected from the abdominal vena cava (< 1 min). Shortly after, a small piece of liver was taken from the median lobe, frozen with aluminium block tongs cooled to liquid nitrogen temperature. Finally the soleus muscle of the right leg was exposed, and a sample of the muscle removed, freeze clamped, and placed in liquid nitrogen. Nonexercised rats were treated in the same manner as the exercised rats and were killed at approximately the same time (40 min).

**ANALYTIC METHODS**

Peripheral blood was collected into 5-cc syringes pretreated with Ethylene Diamine Tetraacetic Acid (7%; EDTA) and immediately separated into two fractions. The first portion of blood (~500 µl) was transferred into tubes containing aprotinin (Trasylol TM; 50 µl), kept in crushed ice, and centrifuged for 2 min (Eppendorf centrifuge, #5415). The plasma was stored for subsequent glucagon determination. The remaining part of blood was also centrifuged for 2 min, and the plasma was stored for subsequent glucose, insulin, C-peptide, free fatty acids (FFA), and β-hydroxybutyrate determinations. All tissues and blood samples were stored at ~78 °C until analyses were performed.

Plasma glucose concentrations were determined by the use of a glucose analyser (Yellow Springs Instruments 2300, Yellow Springs, OH). Insulin and glucagon concentrations were determined by commercially available radioimmunoassay kits using porcine insulin and human glucagon standards (Radioassay System Laboratory: ICN Biomedicals, Costa Mesa, CA; distributed by Immunocorp, Montreal, Quebec). C-peptide was also measured by a commercially radioimmunoassay kit using rat standards (Linco Research, St-Louis, MO). FFA and β-hydroxybutyrate concentrations were assessed by commercially available spectrophotometric methods (reagent kits from Boehringer Mannheim, distributed by Immunocorp). Liver and muscle glycogen concentrations were determined by use of the phenol-sulfuric acid reaction (Lo et al., 1970).

**STATISTICAL ANALYSES**

All data are reported as means ± SE. Statistical comparisons were done using a two-way analysis of variance (ANOVA) for nonrepeated-measures design. Tukey’s post hoc test was used in the event of a significant (P < .05) F-ratio.
Results

Body weights at sacrifice were similar in all groups (X ± SE: 325 ± 2 g; n = 81). Resting (0 min) liver glycogen concentrations were significantly (P < .01) higher in normally fed rats than in the two other groups (Figure 1A). However, no significant differences in resting liver glycogen content were found between 1/2-fast and glucagon-injected rats. Exercise (30 and 50 min) resulted in a significant (P < .01) decrease in liver glycogen concentrations in the normally fed group. Although a tendency for liver glycogen concentrations to decrease following exercise was also observed in 1/2-fast and glucagon-injected rats, the decrease did not reach significant levels. However, liver glycogen concentration was significantly higher

Figure 1. Liver glycogen, plasma glucose, and muscle glycogen concentrations at rest and after 30 and 50 min of exercise in normally fed, 1/2-fast, and glucagon-injected conditions. Values are means ± SE; n = 7–9 rats/group with the exception of values of liver glycogen, at rest, in glucagon-injected group; n = 6 rats. *significantly different from corresponding time in the two other groups, P < .01; **significantly different from rest, P < .01; ***significantly different from time 50 in 1/2-fast group, P < 0.01.
at the end of the 30-min exercise period in the normally fed group than after 50 min of exercise in the 1/2-fast group \( (P < .01) \) and in the glucagon-injected group \( (P < .06) \). Blood glucose concentrations were not affected by either the pre-experimental manipulations or the exercise protocol (Figure 1B). Glycogen concentrations in the soleus muscle were significantly \( (P < .01) \) decreased following 30 and 50 min of exercise in the three conditions (Figure 1C). There were, however, no significant \( (P > .05) \) intergroup differences at any time.

Plasma insulin concentrations were significantly \( (P < .01) \) decreased after 50 min of exercise in the three groups (Figure 2A). On the whole, the plasma insulin concentrations were significantly \( (P < .01) \) higher in the glucagon-injected

![Figure 2](image.png)  
**Figure 2.** Plasma insulin, C-peptides, and glucagon concentrations at rest and after 30 and 50 min of exercise in normally fed, 1/2-fast, and glucagon-injected conditions. Values are means ± SE; \( n = 8–9 \) rats/group; *significantly different between indicated groups, \( P < .01 \); **significantly different from rest, \( P < .01 \); ***significantly different from time 30, \( P < .05 \).
than in the 1/2-fast groups. A similar tendency was also found \( (P < .08) \) between insulin values in normally fed and 1/2-fast groups. No significant differences in plasma C-Peptides were found between the three conditions (Figure 2B). However, at the end of the 50-min exercise period, C-Peptides values were significantly decreased compared to rest \( (P < .01) \) and the 30-min exercise values \( (P < .05) \) in the three groups. Plasma glucagon concentrations in the three experimental groups were not significantly \( (P > 0.05) \) affected by exercise (Figure 2C).

Free fatty acid concentrations, following 30 and 50 min of exercise were significantly \( (P < .01) \) increased compared to resting values in all three conditions (Figure 3A). However, a main group effect \( (P < .05) \) was found between normally fed and glucagon-injected groups with higher FFA values measured in the latter.

![Graph A](image1)

![Graph B](image2)

**Figure 3.** Plasma free fatty acid and β-hydroxybutyrate concentrations at rest and after 30 and 50 min of exercise in normally fed, 1/2-fast, and glucagon-injected conditions. Values are means ± SE; \( n = 8–9 \) rats/group. *significantly different from rest, \( P < .01 \), \( P < .05 \); **significantly different between indicated groups, \( P < .05 \); ***significantly different from time 50 in the two other groups, \( P < .01 \).
The same comparison between 1/2-fast and normally fed groups was found to be significant ($P < .08$). However, no significant differences in FFA concentrations were found between 1/2-fast and glucagon-injected groups ($P > 0.05$). No significant differences ($P > 0.05$) were found in resting β-hydroxybutyrate concentrations between the three groups (Figure 3B). Exercise (50 min) resulted in a significant ($P < .01$) increase in β-hydroxybutyrate concentrations in 1/2-fast and glucagon-injected rats ($P < .05$). At the end of the 50-min exercise period, β-hydroxybutyrate concentrations were similar in normally fed and glucagon-injected rats, the two of them being significantly ($P < .01$) lower than corresponding values measured in 1/2-fast rats (Figure 3B).

Discussion

Resting hepatic glycogen content was approximately 50% lower in 1/2-fast and glucagon-injected rats compared to normally fed rats. This indicates that both of these manipulations were successful in creating a substantial reduction in liver glycogen levels before exercise. It is also important to observe that the liver glycogen concentration reached, following the present glucagon injections, was in the same range as the one measured following the food intake reduction ($X \pm SE$: 1.1 ± 0.1 vs. 0.93 ± 0.1 g/100g). This was done on purpose using the same glucagon injection protocol as the one used in one of our recent studies (Bélanger et al., 1998) for which similar results had been obtained. The present glycogen data, therefore, clearly show that similar reduced hepatic glycogen contents prior to exercise were measured in 1/2-fast and glucagon-injected rats as a result of two different manipulations. During exercise, liver glycogen content was significantly reduced only in the normally fed rats, reaching levels, after 50 min of exercise, similar to the one measured in the two other groups. The absence of a significant exercise reduction in liver glycogen in 1/2-fast and glucagon-injected groups is most likely related to the low initial liver glycogen concentration. Most importantly, however, these results show that the reduction in liver glycogen content during exercise was twice as great in the normally fed than in 1/2-fast and glucagon-injected rats ($X_1$: 1.53 vs. 0.61 and 0.58 g/100 g, respectively). Accordingly, liver glycogen content after 30 min of exercise was higher in normally fed rats than after 50 min in the two other groups. This indicates that during the course of the exercise period, the liver glycogen content was higher in the normally fed rats than it was in the two other groups most of the time.

Although initial liver glycogen content was reduced in two of the three groups, this did not result in a decrease in blood glucose concentrations either at rest or after exercise. This indicates that the effect of the dietary and glucagon injection manipulations on liver glycogen reserve was not profound enough to disturb blood glucose homeostasis. On the other hand, exercise (50 min) resulted in a larger increase in β-hydroxybutyrate concentrations in the 1/2-fast group than in the two other groups (Figure 3B). Although no significant intergroup differences in resting β-hydroxybutyrate concentrations were measured, the higher β-hydroxybutyrate levels found after 50 min of exercise in 1/2-fast rats can be interpreted as the result of a combined effect of the food reduction and the exercise stimuli. It is interesting to observe that although plasma FFA concentrations were increased to a similar extent during exercise in the 1/2-fast and glucagon-injected rats, β-hydroxybutyrate
concentrations during exercise were twice as high in 1/2-fast than in glucagon-injected rats. This indicates that the greater activation of hepatic ketogenesis in 1/2-fast rats was not solely related to the plasma FFA availability. These data may, therefore, be taken as an indication that indeed a state of food restriction caused some specific metabolic perturbations that were not present in the glucagon-injected rats.

The main finding of the present study is that the increase in FFA concentrations normally observed during exercise was more pronounced in the glucagon-injected than in the normally fed groups, the same comparison between the 1/2-fast and the normally fed groups showing a strong tendency ($P < .08$) to higher values in the former. It is well-known that a reduction in food intake is associated with a larger increase in FFA mobilization during exercise (Galbo et al., 1979; Jansson et al., 1982). The exact nature of the stimulus for such metabolic adaptation to food intake reduction or low carbohydrate diet during exercise is still not fully understood. Potential influential factors are a reduction in blood glucose level (Galbo et al., 1977), tissue glycogen content (muscle and/or liver; Koslowski et al., 1981; Lavoie et al., 1984), pre-exercise insulin (Galbo et al., 1981), and possibly the increase in ketone bodies concentration (Robinson and Williamson, 1980). In recent years, our laboratory has gathered some evidence suggesting that the decreasing level of liver glycogen during exercise may be a factor that afferently stimulates lipid mobilization (Hélie et al., 1985; Lavoie et al., 1987). The problem with the approach used in these studies, as well as in more recent ones (Welsh et al., 1998a, 1998b), is that liver and/or muscle glycogen decrease can not be totally differentiated of other metabolic effects caused by a reduction in food intake, even though euglycemia was maintained. For instance, FFA and insulin concentrations, respectively, may be increased and decreased at the beginning of the exercise period. The interest of the present approach was that a decrease in liver glycogen before exercise was obtained without using any food restriction protocol. Since the reduction of liver glycogen with the glucagon injections was only partial, there were no noticeable changes in resting and exercising blood glucose concentrations. The present increase in exercising FFA concentration in glucagon-injected rats, therefore, suggests that the low hepatic glycogen concentrations, independently of any effects of a reduction in food intake, stimulated FFA mobilization during exercise. The stimulation of FFA mobilization by the low liver glycogen concentrations may be brought about by an increase in sympathetic activation. Unfortunately, we had technical problems in measuring plasma catecholamines in the present study. In a previous study (Lavoie et al., 1989), however, plasma norepinephrine concentrations have been shown to be reduced by a hepatic vagotomy in adrenomedullated rats, suggesting that the liver, and most likely its glycogen content, is able to influence the sympathetic response to exercise.

The concept that a low liver glycogen content, as such, may act as a metabolic regulator in different situations, such as exercise, has received a fair amount of attention in the last few years (Lavoie and Cardin, 1996). Bernal and coworkers (1982), for one, had reported that a small intraportal infusion of glucose lowered plasma FFA; however, this effect was not seen when glucose was infused into a peripheral vein. The hepatic afferent concept implies that the liver is able to afferently inform the central nervous system or other organs about changes related to a decreasing liver glycogen content. This information would contribute to the
stimulation of a compensatory response, such as an increase in FFA mobilization and indirectly utilization, that will help prevent a rapid decrease in blood glucose levels. The present results are in line with this concept and show for the first time that the relation between a decreasing liver glycogen content and the increase in FFA mobilization during exercise may be dissociated from a state of nutritional deficiency.

There are other situations where an increase in plasma FFA levels is associated with a decrease in liver glycogen content. Partial hepatectomy, for instance, is a situation where the remnant liver shows a substantial decrease in liver glycogen and a large increase in plasma FFA without any noticeable changes in blood glucose concentrations (Lavoie et al., 1998; Nakatani et al., 1981). It has been postulated that these metabolic changes are linked to the decreased energy charge level (i.e., liver ATP levels; Nakatani et al., 1981). The same phenomena of an increased lipid mobilization is also observed when ethionine is administrated, causing a rapid decrease in liver glycogen and ATP concentrations, in presence of a mild hypoglycemia (Lyon and Kisilevsky, 1986; Tani et al., 1973). Based on these reported observations, it can be speculated that a decrease in liver glycogen during exercise, possibly associated with a decrease in liver ATP levels (Dohm and Newsholme, 1983; Ghanbari-Niaki et al., 1999), may afferently stimulate an increase in lipid mobilization, indirectly an increase in lipid utilization, and in this way contribute to the maintenance of blood glucose levels.

Besides a reduction in liver glycogen content, there are other possibilities to explain the higher lipid mobilization in the glucagon-injected group that need to be addressed. Insulin concentrations were, on the whole, slightly higher in the glucagon-injected rats than in the 1/2-fast group. This, however, does not constitute an alternative stimulus to explain the higher plasma FFA concentrations in the glucagon-injected rats, since insulin is known to inhibit FFA mobilization. It might also be argued that the somewhat larger reduction from rest to exercise in insulin and C-peptide concentrations in the glucagon-injected rats may have triggered the larger increase in plasma FFA concentrations observed in this group as compared to the normally fed rats. One has to take into account, however, that there were no significant differences between groups in insulin and C-peptide concentrations during exercise. On the other hand, we previously reported (Lavoie et al., 1989) that a hepatic vagotomy attenuates the exercise-induced reduction in insulin response. Based on this observation, the present exercise situation should have resulted in lower insulin concentrations in the low liver glycogen groups. The discrepancy between the two studies may be explained by the fact that in our previous study (Lavoie et al., 1989), rats were adrenomedullated to release the inhibitory effect of epinephrine on insulin secretion. There were no significant differences in resting muscle glycogen between the different groups, and muscle glycogen utilization during exercise was similar in all groups. This rules out the possibility that a larger reduction in muscle glycogen utilization may be responsible for the increased FFA mobilization in the glucagon-injected rats.

In summary, data of the present experiment show that a low initial liver glycogen content, brought about by a series of glucagon injections, results in a higher plasma FFA concentration during a subsequent period of exercise. These results are the first to differentiate a reduction in liver glycogen content from the general effects of a reduction in food or carbohydrate intake as a metabolic regulator acting during exercise.
References


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