Alterations in hepatic glucagon receptor density and in Gsα and Giα2 protein content with diet-induced hepatic steatosis: effects of acute exercise

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Am J Physiol Endocrinol Metab 289: E8–E14, 2005. First published February 1, 2005; doi:10.1152/ajpendo.00570.2004. —The present study was undertaken to test the hypothesis that a high-fat diet-induced liver lipid infiltration is associated with a reduction of hepatic glucagon receptor density (Bmax) and affinity (K0), and with a decrease in stimulatory G protein (Gsα) content while enhancing inhibitory G protein (Giα2) expression. We also hypothesized that, under this dietary condition, a single bout of endurance exercise would restore hepatic glucagon receptor parameters and G protein expression to standard levels. Female Sprague-Dawley rats were fed either a standard (SD) or a high-fat diet (HF; 40% kcal) for 2 wk (n = 20 rats/group). Each dietary group was thereafter subdivided into a nonexercised (Rest) and an acute-exercised group (Ac-Ex). The acute exercise consisted of a single bout of endurance exercise on a treadmill (30 min, 26 m/min, and 0% slope) immediately before being killed. The HF compared with the SD diet was associated with significantly (P < 0.05) higher values in hepatic triglyceride concentrations (123%), fat pad weight, and plasma free fatty acid (FFA) concentrations. The HF diet also resulted in significantly (P < 0.05) lower hepatic glucagon receptor density (45%) and Gα protein content (75%), as well as higher (P < 0.05) Gα protein content (27%), with no significant effects on glucagon receptor affinity. Comparisons of all individual liver triglyceride and Bmax values revealed that liver triglycerides were highly (R < 0.003) predictive of the decreased glucagon receptor density (R = -0.512). Although the 30-min exercise bout resulted in some typical exercise effects (P < 0.05), such as an increase in FFA (SD diet), a decrease in insulin levels, and an increase in plasma glucagon concentrations (SD diet), it did not change any of the responses related to liver glucagon receptors and G proteins, with the exception of a significant (P < 0.05) decrease in Gα2 protein content under the HF diet. The present results indicate that the feeding of an HF diet is associated with a reduction in plasma membrane hepatic glucagon receptor density and Gα protein content, which is not attenuated by a 30-min exercise bout. It is suggested that liver lipid infiltration plays a role in reducing glucagon action in the liver through a reduction in glucagon receptor density and glucagon-mediated signal transduction.

liver lipid infiltration; glucagon receptor affinity; fat deposits; high-fat diet; liver
endurance training increases hepatic glucagon receptor density and the G/G ratio in rats. There is also evidence that not only chronic but also acute exercise (60 min) may enhance hepatic glucose production in response to glucagon (28). Whether hepatic glucagon receptor density and G protein content are also increased by an acute bout of exercise is unknown. Therefore, the present study was also designed to test the hypothesis that an acute bout of exercise restores hepatic glucagon receptor density and G protein content under the condition of high-fat diet-induced hepatic lipid infiltration.

METHODS

Animal care and exercise protocol. Female Sprague-Dawley rats (Charles River, St. Constant, QC, Canada), weighing 180–200 g, were housed individually and allowed food [standard (SD) or high-fat (HF) diet] and water ad libitum for 2 wk after they were received in our laboratory. The lighting schedule was such that lights were on from 0700 until 1900, and room temperature was maintained at 20–23°C. Seven days after their arrival, rats assigned to acutely exercised groups underwent a habituation running protocol on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA) consisting of three sessions over a 7-day period, beginning with 15 min/day at 20 m/min, followed by a progressive increase to 30 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 days before the experiment for all rats. All exercised animals were restrained from exercise 48 h before the experimentation.

Groups and surgery. After their arrival, rats were randomly assigned to one of the four experimental groups. One group of rats was fed with SD pellet rat chow (12.5% lipids, 62.3% carbohydrate, and 24.3% protein (kcal); Agribbrands Purina Canada, Woodstock, ON, Canada). A second group was fed with HF small pellet rat chow (42% lipids, 80% lard, 20% corn oil, 36% carbohydrate, and 22% protein (kcal); ICN Pharmaceuticals, New York, NY). The duration of the diet (2 wk) was based on previous time course studies (13) in which we observed that hepatic triglyceride content was highest 2 wk after the undertaking of an HF diet. Details of the diets are described elsewhere (14). One-half of the animals in each dietary condition were exercised (Ac-Ex) prior to the experimentation (30 min at 26 m/min, 0% grade), whereas the other half remained inactive (Rest). Hence the four experimental groups consisted of SD-fed rats with no exercise (SD/Rest) or acute exercise (SD/Ac-Ex), and HF-fed rats with no exercise (HF/Rest) or acute exercise (HF/Ac-Ex), with n = 10 rats/group. On the morning of the experiment, any remaining food was removed from the cages 2–3 h before the rats were killed. Rats were weighed and anesthetized with pentobarbital sodium (40 mg/kg ip). After complete anesthesia, the abdominal cavity was rapidly opened, following the median line of the abdomen. Blood was rapidly (<45 s) drawn from the abdominal vena cava (~4 ml) into a syringe pretreated with EDTA (15%). The fraction of blood (250 μl) to be used for glucagon determination was preserved in aprotinin (25 μl) before centrifugation. The remaining fraction of blood was also centrifuged (Eppendorf centrifuge no. 5415), and the plasma was stored for subsequent plasma glucose, insulin, triglyceride, and free fatty acid (FFA) determinations. The liver and the mesenteric, urogenital, and retroperitoneal fat deposits were excised and weighed in that order. The liver median lobe was freeze-clamped and used for glycerogen and triacylglycerol determinations, and 4 g of fresh liver were taken for membrane isolation. Mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastroepiploic sphincter to the end of the rectum with special care taken in distinguishing and removing pancreatic cells. Urogenital fat pad included adipose tissue surrounding the kidneys, ureters, and bladder as well as ovaries, oviducts, and uterus. Retroperitoneal fat pad was taken as that distinct deposit behind each kidney, along the lumbar muscles. All plasma and tissue samples were stored at −78°C until analyses.

Materials and isolation of plasma membranes. Sucrose (ultrapure) was obtained from Schwarz/Mann Div., Becton-Dickinson (Orangeburg, NY); p-nitrophenyl thymidine mono-phosphate, cytochrome c, glucose 6-phosphate (Na salt), alkaline phosphatase substrate, p-nitrophenyl-β-N-acetyl-glucosaminide, agarose, and Tris base from Sigma Chemical (St. Louis, MO); protein reagent from Bio-Rad Laboratories (Richmond, CA); 5'-adenosine monophosphate (5'-AMP), NADPH, NADH, and ferritin (A grade) from Calbiochem-Behring (American Hoescht, San Diego, CA); N,N-dimethyl-1,3-propane diamine from Eastman Organic Chemicals Div., Eastman Kodak CO (Rochester, NY), glutaraldehyde and osmium tetroxide from Electron Microscopy Sciences (Fort Washington, PA); and 1-ethyl-3-(3-dimethylaminopropyl)-N'-carbodiimide hydrochloride from Ott Chemical (Muskegan, MI). All other chemicals were reagent grade. All sucrose solutions were prepared 24–48 h before use and their densities determined at room temperature with an Abbé refractometer. The sucrose solutions were filtered (0.22 μm for 0.25 M and 1.2 μm for 1.42 and 2.0 M solutions), the pH and density determined and stored at 4°C.

Liver samples (1.8–2 g) were weighed, added to 10 volumes of 0.25 M sucrose in a 15-ml glass disposable culture tube (0.25 M sucrose-5 mM Tris-HCl, pH 7.2–7.6, and 0.5 and 1.0 mM MgCl2), and homogenized with a Polytron (Polyscience, model X-520) at 1,000–1,100 rpm. The solution was centrifuged (25–30 ml/50 ml plastic tube) at 280 g or 1,800 rpm for 5 min (Beckman GPR centrifuge). The supernatant was saved, and the pellet resuspended in 0.25 M, a volume corresponding to one-half the initial homogenate volume. The suspension was again centrifuged as above. The first and second supernatants were combined and centrifuged at 1,500 g or 2,600 rpm for 10 min. The resulting pellets were pooled and resuspended in 1–2 ml of 0.25 M sucrose/g liver (initial wet weight). Sucrose (2 mM) was added to obtain a density of 1.18 g/cm³ (1.42 M, n = 1,401), and sufficient sucrose was added to bring the volume to approximately twice that of the original homogenate (i.e., 10% wt/vol). Aliquots (35 ml) of the sample were added to cellulose nitrate tubes and overlaid with 2–4 ml of 0.25 M sucrose. After centrifugation for 60 min at 82,000 g (25,000 rpm) in a Beckman ultracentrifuge (model L5–50), the pellet at the interface was collected with a blunt-tipped Pasteur pipette and resuspended in 0.25 M sucrose to obtain a density of 1.05 g/cm³. This suspension was centrifuged at 1,500 g for 10 min, and the final pellet was resuspended in 50 mM HEPES and stored at −78°C for further analysis.

Binding assay. The receptor binding assay used is based on the technique described by Frandsen et al. (12) and as modified by Légaré et al. (25). Purified membranes (10 μg proteins/150 μl), in triplicate, were incubated with 125I-labeled glucagon (NEN Life Science Products, Boston, MA) at concentrations ranging from 0.01 to 2.0 nM in HEPES buffer (50 mM, pH 7.6) containing 1% human serum albumin (HAS; Sigma-Aldrich). Incubations were carried out at 30°C for 30 min in a total volume of 150 μl. Aliquots of 100 μl were added to microfuge tubes containing 200 μl of cold (4°C) 2.5% HAS in HEPES buffer (50 mM, pH 7.6). Free and membrane-bound [125I]glucagon were separated by centrifugation at 10,000 g for 5 min, and the supernatant was discarded. The membrane pellet was washed once with 200 μl of cold HEPES buffer containing 2.5% HAS, and the membrane-bound radioactivity was determined with the use of a gamma counter (Wallac 1470 Wizard, Wellesley, MA). The nonspecific binding was measured in the presence of 3 × 10−6 M glucagon (Sigma-Aldrich). On the basis of preliminary experiments, an incubation time of 30 min with membrane protein concentration of 10 μg/150 μl was selected to ensure saturation kinetic sensitivity. The maximal
Mesenteric fat pad, g/100 g BW 3.0

day 14,

Weight at

followed by a 2-min immersion in near-boiling water.

Liver membrane lysates containing 100 supernatant was measured using a Bradford protein assay (Bio-Rad).

fuged at 4,500 g

065-003; Jackson Laboratory). To quantify hepatic Gs rabbit anti-goat IgG conjugated to horseradish peroxidase (no. 305- antibodies (Calbiochem, La Jolla, CA), and then identified using

branes, incubated with purified polyclonal rabbit antisera primary samples were electrophoresed, transferred to nitrocellulose mem-

munoblotting techniques, as previously described (17). Briefly, all

density (Bmax) and the apparent affinity (Kd) of glucagon receptors were obtained through rectangular hyperbolic regression of the specific binding curve (GraphPad Software, San Diego, CA).

Preparation for SDS-PAGE immunoblotting. Liver membrane homo-
mogenates were vortexed frequently for 1 h at 4°C and then centri-
fuged at 4,500 g for 1 h at 4°C. The protein concentration of the supernatant was measured using a Bradford protein assay (Bio-Rad).

Liver membrane lysates containing 100 µg of proteins were prepared for SDS-PAGE by dilutions with reducing sample buffer (Laemmli) followed by a 2-min immersion in near-boiling water.

Quantification of Gsα and Gα2 by immunoblotting. Assessment of the stimulatory and inhibitory α-subunits of the hepatic G proteins (Gsα and Gα2) was conducted using standard SDS-PAGE and immunoblotting techniques, as previously described (17). Briefly, all samples were electrophoresed, transferred to nitrocellulose membranes, incubated with purified polyclonal rabbit antisera primary antibodies (Calbiochem, La Jolla, CA), and then identified using rabbit anti-goat IgG conjugated to horseradish peroxidase (no. 305-065-003; Jackson Laboratory). To quantify hepatic Gsα and to ensure equal lane loading, purified fusion proteins were constructed as previously described (32), and both protein standards and sample bands at 48 and 45 kDa were quantified (two distinct isoforms confirmed by Calbiochem). Similar procedures were performed on the 39-kDa band for assessment of Gα2. All gels were poured so that

samples from each of the experimental groups were always electrophoresed on the same gels to ensure standardized Western blotting analysis. G proteins were visualized with chemiluminescent solutions A and B (Amersham, Alameda, CA). Image capture analysis was performed on the fusion proteins to quantify G protein content of all membrane samples.

Analytic methods. Plasma glucose concentrations were determined using a glucose analyzer (Yellow Springs Intruments 2300, Yellow Springs, OH). Insulin and glucagon concentrations were determined by commercially available radioimmunoassay kits (Radioasay System Laboratory, ICN Biomedicals, Costa Mesa, CA; distributed by Immunocorp, Montreal, QC, Canada). Plasma and liver triglyceride concentrations were determined by quantitative enzymatic method (Sigma Diagnostics, St. Louis, MO). Plasma glucose was determined by enzymatic colorimetric assay (Roche Diagnostics, Laval, QC, Canada). Liver glycogen concentrations were determined using the phenol-sulfuric acid reaction (27).

Statistical analysis. All data are reported as means ± SE. Statistical comparisons were performed using a two-way ANOVA for nonrepeated measures design. The Newman-Keuls post hoc test was used in the event of a significant (P < 0.05) F ratio. Relationship between liver triglyceride concentration and glucagon receptor density (Bmax) was evaluated by linear regression analysis.
RESULTS

There was no difference in body weight between groups at any measured points in time during the 2-wk experimental period (Table 1). Nevertheless, the feeding of the HF diet resulted in a significantly higher relative weight of mesenteric, retroperitoneal, and urogenital fat deposits ($P < 0.05$). Acute exercise was associated with a significantly ($P < 0.05$) lower weight of all fat pads in the HF-fed group (Table 1). No differences were observed in relative liver weight between the HF- and SD-fed groups. Nonetheless, acute exercise resulted in a significantly ($P < 0.05$) lower liver weight in both SD and HF dietary conditions (Table 1).

No differences in plasma glucose concentration were observed between all groups (Fig. 1). Plasma insulin concentration did not change after the dietary manipulations either in the sedentary or in the acute exercise state (Fig. 1). Nevertheless, exercise resulted in significantly ($P < 0.05$) lower plasma insulin concentrations in both SD- and HF-fed groups. Dietary manipulations did not significantly ($P > 0.05$) alter plasma glucagon concentration (Fig. 1). However, exercise resulted in a significant ($P < 0.05$) increase in plasma glucagon concentration in the SD-fed group.

As expected, hepatic triglyceride concentrations were significantly ($P < 0.01$) higher with the feeding of the HF diet (Fig. 2). Liver triglyceride levels were not affected by the acute exercise in either dietary protocol. Despite a lack of statistical significance, hepatic glucagon concentrations were slightly lower in the HF- compared with the SD-fed rats in both sedentary and exercised groups (Fig. 2). There was also a tendency to lower glucagon levels after the acute exercise in both dietary conditions. Similar to hepatic triglycerides, plasma FFA concentrations were significantly ($P < 0.05$) higher in the HF- compared with the SD-fed group in the resting state (Fig. 2; $P < 0.05$). Exercise resulted in a significant ($P < 0.05$) increase in plasma FFA concentrations in the SD-fed group only. The HF diet did not affect plasma triglyceride concentrations in the resting state. However, a single bout of endurance exercise resulted in significantly higher plasma triglyceride concentrations in the HF-fed rats (Fig. 2; $P < 0.05$). This resulted in higher ($P < 0.05$) plasma triglyceride concentra-
In agreement with our hypotheses, glucagon receptor density ($B_{\text{max}}$) was significantly ($P < 0.01$) reduced with the feeding of an HF diet (Fig. 3). An $\sim 45\%$ decrease in $B_{\text{max}}$ was observed in both HF-fed groups. Acute exercise, however, did not have any significant ($P > 0.05$) effect on glucagon receptor density. No differences in glucagon receptor affinity ($K_{d}$) were observed between any of the groups (Fig. 3). Hepatic $G_{\alpha}$ content in rats fed the HF diet was $\sim 75\%$ ($P < 0.05$) lower than in rats fed the SD diet in both rest and acute exercise conditions (Fig. 4). The acute exercise bout had no effect on liver $G_{\alpha}$ content. There was an $\sim 27\%$ ($P < 0.05$) increase in $G_{\alpha_2}$ content with the feeding of the HF diet in the resting state. The acute exercise bout significantly ($P < 0.05$) reduced $G_{\alpha_2}$ content under the HF diet (Fig. 4). Even though $G_{\alpha_2}$ content was increased in the HF/Rest group, the $G_{\alpha}/G_{\alpha_2}$ ratio was $\sim 80\%$ lower in the HF- compared with the SD-fed groups (Fig. 4). Acute exercise had no effect on the $G_{\alpha}/G_{\alpha_2}$ ratio.

Figure 5 shows the association between the level of hepatic triglycerides and the $B_{\text{max}}$ for all rats throughout the experiment. This comparison reveals a negative relationship between glucagon receptor density and hepatic triglyceride concentrations ($R = -0.512; P < 0.003$).

**DISCUSSION**

The present HF diet regimen in rats resulted in a 123% higher fat accumulation in liver compared with the SD-fed animals (Fig. 2). This indicates that the present nutritional manipulations were adequate to cause a substantial accretion of fat inside the liver that is compatible with the development of a state of hepatic steatosis (13, 14, 37). In addition to liver lipid infiltration, the present HF diet resulted in a threefold increase in plasma FFA concentration. Hepatic steatosis is often accompanied with an increase in plasma FFA levels (reviewed in Ref. 26). In previous studies in which hepatic steatosis was induced by HF diets, a substantial increase in plasma FFA was always observed (13, 14, 37). Taken together, the increased plasma FFA levels following the present HF diet are consistent with a metabolic situation leading to the development of a state of hepatic steatosis.

The major finding of the present investigation is that an HF diet-induced liver lipid infiltration is associated with a 45% reduction in glucagon receptor number ($B_{\text{max}}$), accompanied by a 75% lower plasma membrane level of $G_{\alpha}$ protein.

**Fig. 4.** Stimulatory and inhibitory $\alpha$-subunits of hepatic $G$ proteins ($G_{\alpha}$ and $G_{\alpha_2}$) and the $G_{\alpha}/G_{\alpha_2}$ ratio in Rest and Ac-Ex rats after 2 wk of an SD or HF diet. Values are means ± SE; $n = 8–10$ rats/group. *Significantly different between the SD- and HF-fed groups, $P < 0.05$; †Significantly different between Rest and Ac-Ex groups, $P < 0.05$. ‡Significantly different between Ac-Ex groups, $P < 0.05$.

**Fig. 5.** Relationship between glucagon receptor density ($B_{\text{max}}$) and liver triglyceride concentrations ($n = 32$, $P < 0.003$) for all rats in all dietary and rest-exercise conditions.

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content, and a lower G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} ratio. In a previous study (7), we suggested that liver lipid infiltration was associated with a reduction in glucagon action on hepatic glucose production. The present data extend this observation by being the first to indicate a reduction in glucagon receptor density and G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} ratio in an in vivo HF model of hepatic steatosis in rats.

There are several factors that may cause a reduction of glucagon receptor density in the liver. In past studies a decreased number of glucagon receptors has been associated with changes in glucagon (3, 9), glucose, and insulin concentrations (1, 5, 34, 38). Because none of these factors is significantly altered by the present HF diet, these regulators can hardly explain the present HF diet-induced reduction in glucagon receptor density. Data from the present study do suggest, however, that factors related to the HF diet-induced hepatic steatosis are involved in the reduction in glucagon receptor number. It is not clear, however, whether the present HF diet-induced reduction in glucagon receptor density and in G\textsubscript{\alpha} protein content is due to systemic factors (i.e., circulating lipids) or to elevated intrahepatic triglycerides. In a recent study conducted on isolated livers from HF-fed rats, it was concluded that hepatic insulin resistance observed in vivo may at least partially result from circulating factors (4). Plasma FFA levels were largely increased by the present HF diet and may, at least partly, explain the reduction in glucagon receptor density. On the other hand, Samuel et al. (36) recently used a 3-day HF diet model to increase liver triglyceride content (3-fold), without the confounding effects of peripheral fat accumulation. They provided evidence to support the causal relationship between hepatic fat accumulation and hepatic insulin resistance. That intrahepatic factors (liver triglycerides) regulate glucagon receptor density is supported by the negative association found between the level of hepatic triglycerides and the measurement of glucagon receptor density. A similar negative association was observed in our previous study between glucagon-stimulated hepatic glucose production and hepatic triglyceride concentrations (7). On the other hand, glucagon receptor sensitivity was not affected by the present HF diet. This is in line with the findings of Légare et al. (25), who reported an increase in glucagon receptor density but no difference in the affinity of the glucagon receptors in trained vs. untrained rats. Taken together, the present data indicate that an HF diet-induced lipid infiltration in the liver results in a reduction in hepatic plasma membrane glucagon receptor density and suggest that this may explain the development of a state of hepatic glucagon resistance under these circumstances.

The precise mechanism(s) by which an HF diet and/or hepatic lipids would reduce hepatic glucagon receptor density remains to be elucidated. Fat-induced hepatic insulin resistance has been attributed to impaired insulin-stimulated insulin receptor substrate-1 (IRS-1) and IRS-2 tyrosine phosphorylation associated with activation of some isoforms of PKC (22, 36). Hepatic lipids (phospholipids) have also been shown to be potent activators of G protein-coupled receptor kinases (8, 31). G protein-coupled receptor kinases mediate phosphorylation of G protein-coupled receptors and initiate homologous receptor desensitization (23, 24). Whether the accumulation of hepatic lipids could desensitize the glucagon receptors through activation of such proteins remains to be explored.

The 75% reduction in G\textsubscript{\alpha} content following the HF diet observed in the present investigation is consistent with results found in genetically obese animals. Liver cell membranes from ob/ob and db/db mice contain significantly (45%) less \alpha-subunit of stimulatory G protein than those from their lean littermates (2, 15). However, the present HF diet-induced increase in G\textsubscript{\alpha\_2} content (27%) has not been found in other studies. Begin-Heick (2) and Gettys et al. (15) observed a significant decrease in G\textsubscript{\alpha\_2} protein content in liver plasma membranes of db/db and ob/ob mice. Nevertheless, the G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} ratio was largely decreased by the present HF diet regimen. Podolin et al. (32) reported a reduced protein expression of G\textsubscript{\alpha} and G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} ratio (20%) with aging in rat liver. Because liver triglycerides have been reported to increase with age (29), it is possible that the decreased hepatic ratio of G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} activity observed in these studies was influenced by the liver lipid content. On the whole, the present decrease in protein expression of G\textsubscript{\alpha} and in the G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} ratio indicates that the HF diet-induced hepatic steatosis reduced not only glucagon receptor density but also glucagon-mediated signal transduction.

Rats were submitted to an acute period of exercise in the present study to test the possibility that such an acute activity might alter the number of plasma membrane glucagon receptors and G protein content. In previous studies, exercise training was shown to increase hepatic glucagon receptors (25). We also reported that a 60-min exercise bout largely increased glucagon-induced hepatic glucose production (28). Surprisingly, however, the 30-min acute bout of aerobic exercise used in the present study did not produce any noticeable effects on glucagon receptor numbers, nor did it affect G\textsubscript{\alpha} protein content and G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} ratio. It is possible that the present 30-min exercise bout might not have been long enough to stimulate hepatic changes that facilitate glucagon action. Recent observations from our laboratory indicate that a 3-h bout of swimming exercise in rats resulted in a large increase in receptor numbers and affinity in SD-fed rats (C. Lavoie, unpublished observation). It is not known, however, whether such a long duration of exercise may affect the HF diet-induced reduction in glucagon receptor number. The reduced G\textsubscript{\alpha} protein content after the present acute exercise in HF-fed rats might be taken as an indication that an acute period of exercise may in fact facilitate the action of glucagon under this dietary condition.

In summary, results of the present study indicate that a HF diet-induced liver lipid infiltration results in a reduction in glucagon receptor numbers, G\textsubscript{\alpha} protein content, and G\textsubscript{\alpha}/G\textsubscript{\alpha\_2} ratio. On the basis of the negative association between hepatic triglyceride content and glucagon receptor density, it is suggested that liver lipid infiltration plays a role in the decrease in glucagon receptor number and glucagon-mediated signal transduction in the liver of HF-fed rats. Further studies should consider the possibilities that HF diet-induced hepatic steatosis causes an internalization of glucagon receptors in hepatocytes, as it has been postulated for hepatic insulin receptors (16) or a downregulation of glucagon receptor expression (1).

GRANTS

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REFERENCES


