

Alexandre Charbonneau, Alexandre Melancon, Carole Lavoie and Jean-Marc Lavoie

Am J Physiol Endocrinol Metab 289:8-14, 2005. First published Feb 1, 2005;
doi:10.1152/ajpendo.00570.2004

You might find this additional information useful...

This article cites 36 articles, 21 of which you can access free at:

<http://ajpendo.physiology.org/cgi/content/full/289/1/E8#BIBL>

This article has been cited by 1 other HighWire hosted article:

High-fat diet-induced hepatic steatosis reduces glucagon receptor content in rat hepatocytes: potential interaction with acute exercise

A. Charbonneau, C. G. Unson and J.-M. Lavoie
J. Physiol., February 15, 2007; 579 (1): 255-267.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high-resolution figures, can be found at:

<http://ajpendo.physiology.org/cgi/content/full/289/1/E8>

Additional material and information about *AJP - Endocrinology and Metabolism* can be found at:

<http://www.the-aps.org/publications/ajpendo>

This information is current as of August 13, 2007 .

Alterations in hepatic glucagon receptor density and in $G_s\alpha$ and $G_i\alpha_2$ protein content with diet-induced hepatic steatosis: effects of acute exercise

Alexandre Charbonneau,¹ Alexandre Melancon,² Carole Lavoie,² and Jean-Marc Lavoie¹

¹Département de Kinésiologie, Université de Montréal, Montreal; and ²Département des Sciences de l'Activité Physique, Université du Québec à Trois-Rivières, Trois-Rivières, Quebec, Canada

Submitted 3 December 2004; accepted in final form 28 January 2005

Charbonneau, Alexandre, Alexandre Melancon, Carole Lavoie, and Jean-Marc Lavoie. Alterations in hepatic glucagon receptor density and in $G_s\alpha$ and $G_i\alpha_2$ protein content with diet-induced hepatic steatosis: effects of acute exercise. *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 (B_{max}) and affinity (K_d), and with a decrease in stimulatory G protein ($G_s\alpha$) content while enhancing inhibitory G protein ($G_i\alpha_2$) 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 $G_s\alpha$ protein content (75%), as well as higher ($P < 0.05$) $G_i\alpha_2$ protein content (27%), with no significant effects on glucagon receptor affinity. Comparisons of all individual liver triglyceride and B_{max} values revealed that liver triglycerides were highly ($P < 0.003$) 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 $G_i\alpha_2$ 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 $G_s\alpha$ 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

IT IS WELL ESTABLISHED that glucagon exerts a strong action on hepatocytes to increase liver glucose production (18). A state of hepatic glucagon resistance has been observed in association with several physiopathologies, including liver cirrhosis, diabetes, and malnutrition (19, 20, 30, 35). Several factors have

been identified to explain a decrease in hepatic glucagon sensitivity under these circumstances. Glucagon homologous desensitization, which is defined as a loss of sensitivity of the glucagon receptor induced by glucagon itself, is one of the plausible explanations. Indeed, under glucagon stimulation, a reduction of liver glucagon receptor density in diabetic rats has been shown in past studies (3, 9). There is also in vivo and in vitro evidence of an alteration in the expression of liver glucagon receptor mRNA by glucose, cAMP, and oxygen (1, 5, 21, 34, 38). Notwithstanding these mechanisms, it is interesting to note that several of the physiopathological conditions associated with a state of hepatic glucagon resistance are also associated with an increase in liver lipid infiltration. In a recent study (7), we observed a reduction in hepatic glucose production in response to glucagon infusion in high-fat diet-induced hepatosteatotic rats. It was suggested that an increase in liver triacylglycerols could interfere with normal hepatic glucagon action. Thus the first purpose of the present study was to extend these observations by testing the hypothesis that a high-fat diet-induced hepatic steatosis is associated with changes in glucagon receptor density and affinity.

There is reported evidence of a disproportionately larger decrease in cAMP response compared with the decrease in hepatic glucagon binding with diabetic hyperglucagonemia (3). This suggests problems may exist in the transfer of membrane receptor signals to the enzyme (33). Obesity and diabetes have been shown to decrease the two main G proteins of the liver membrane [stimulatory ($G_s\alpha$) and inhibitory ($G_i\alpha_2$) (2, 6)]. In addition, Podolin et al. (32) reported that the age-related decline in glucagon signaling capacity and responsiveness may be attributed, in part, to a reduction in the G_s -to- G_i ratio. Obesity, diabetes, and aging (29) are conditions that very often result in an increase in liver lipid infiltration. Whether an increased liver lipid infiltration may result in an alteration in the G protein content in the liver is unknown. Therefore, the second aim of this study was to test the hypothesis that, in addition to a reduction in glucagon receptor density, liver lipid infiltration is also associated with a reduction in hepatic G protein content.

Conversely to several pathophysiological situations (3, 9, 19, 20, 30, 35), chronic and acute physical exercise have been associated with an increased glucagon action in liver. Drouin and colleagues (10, 11) reported that, under glucagon stimulation, trained individuals and animals depicted higher hepatic glucose production than sedentary counterparts. Similarly, Légaré et al. (25) and Podolin et al. (32) demonstrated that

Address for reprint requests and other correspondence: J.-M. Lavoie, Département de Kinésiologie, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montreal, QC, Canada, H3C 3J7 (e-mail: jean-marc.lavoie@umontreal.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

endurance training increases hepatic glucagon receptor density and the G_s/G_i 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); Agribrands 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 μ l) to be used for glucagon determination was preserved in aprotinin (25 μ l) 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 glycogen 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 gastroesophageal 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 (Muskegon, 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 μ m for 0.25 M and 1.2 μ m 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 MgCl_2]), 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 was 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 pellicle 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 μ g proteins/150 μ l), in triplicate, were incubated with ¹²⁵I-labeled glucagon (NEN Life Science Products, Boston, MA) at concentrations ranging from 0.15 to 3.00 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 μ l. Aliquots of 100 μ l were added to microfuge tubes containing 200 μ l 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 μ l 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 μ g/150 μ l was selected to ensure saturation kinetic sensitivity. The maximal

Table 1. BW measured at days 1, 7, and 14 of the experimental period and relative weight of fat pads and liver measured at the end of the experiment

	SD/Rest	SD/Ac-Ex	HF/Rest	HF/Ac-Ex
Weight upon arrival, g	186 ± 1.3	189 ± 2.2	191 ± 2.2	186 ± 1.7
Weight at day 7, g	215 ± 2.4	217 ± 4.9	222 ± 2.0	215 ± 3.2
Weight at day 14, g	239 ± 3.2	240 ± 5.2	250 ± 4.1	244 ± 4.9
Mesenteric fat pad, g/100 g BW	3.0 ± 0.2	3.7 ± 0.2	5.6 ± 0.1*	3.9 ± 0.2†
Retroperitoneal fat pad, g/100 g BW	1.9 ± 0.2	1.7 ± 0.03	3.6 ± 0.2*	2.3 ± 0.2*†
Urogenital fat pad, g/100 g BW	4.4 ± 0.2	4.2 ± 0.2	8.6 ± 0.3*	6.0 ± 0.4*†
Liver weight, g/kg BW	40.0 ± 1.2	37.6 ± 1.0†	38.4 ± 1.4	34.9 ± 0.8†

Values are means ± SE ($n = 8 - 10$ rats/group). BW, body weight; SD/Rest, standard diet/rest; SD/Ac-Ex, standard diet/acute exercise; HF/Rest, high-fat diet/rest; HF/Ac-Ex, high-fat diet/acute exercise. *Significantly different from standard-fed counterpart ($P < 0.05$). †Significantly different between Rest and Ac-E of groups ($P < 0.05$).

density (B_{max}) and the apparent affinity (K_d) 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 homogenates were vortexed frequently for 1 h at 4°C and then centrifuged 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 $G_s\alpha$ and $G_i\alpha_2$ by immunoblotting. Assessment of the stimulatory and inhibitory α -subunits of the hepatic G proteins ($G_s\alpha$ and $G_i\alpha_2$) 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 $G_s\alpha$ 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 $G_i\alpha_2$. 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 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, QC, Canada). Plasma and liver triglyceride concentrations were determined by quantitative enzymatic method (Sigma Diagnostics, St. Louis, MO). FFA concentrations were determined by enzymatic colorimetric assay (Roche Diagnostics, Laval, QC, Canada). Liver glycogen concentrations were determined using the phenolsulfuric 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 (B_{max}) was evaluated by linear regression analysis.

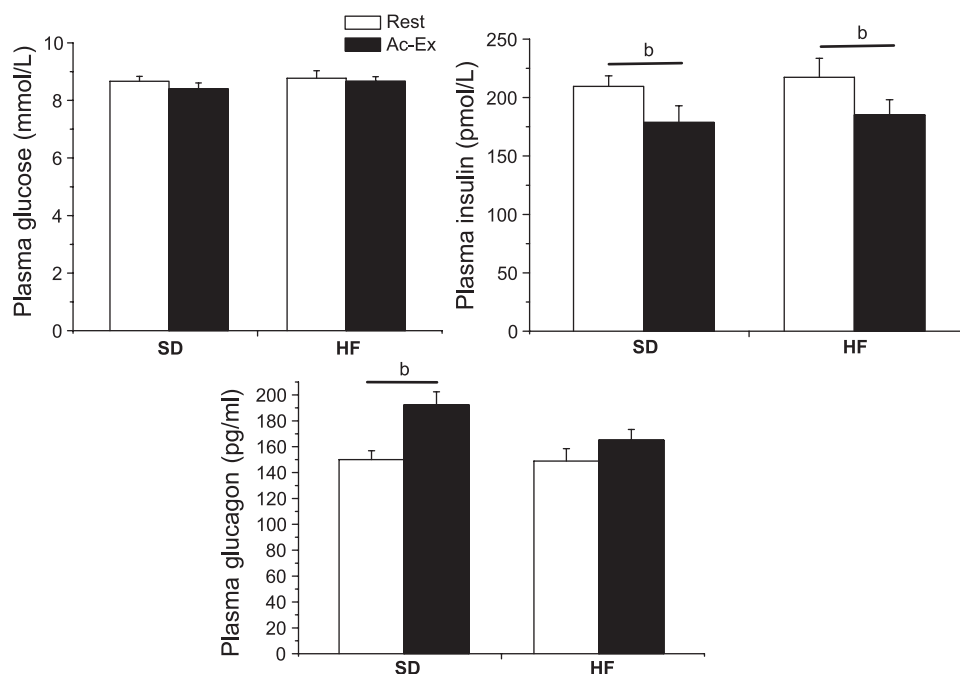


Fig. 1. Plasma glucose, insulin, and glucagon concentrations in resting (Rest) and acutely exercised (Ac-Ex) rats after 2 wk of a standard (SD) or high-fat (HF) diet. Values are means ± SE; $n = 8 - 10$ rats/group. ^bSignificantly different between Rest and Ac-Ex groups, $P < 0.05$.

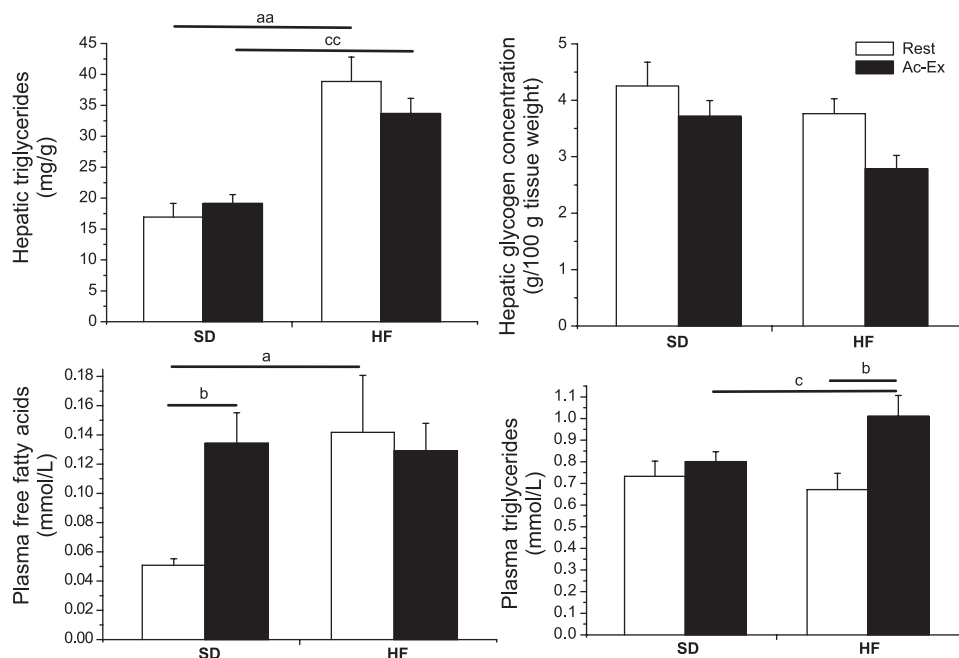


Fig. 2. Hepatic triglyceride and glycogen concentrations and plasma free fatty acid and triglyceride levels in Rest and Ac-Ex rats after 2 wk of an SD or HF diet. Values are means \pm SE; $n = 8-10$ rats/group. ^aSignificantly different between SD- and HF-fed groups, $P < 0.05$; ^{aa} $P < 0.01$. ^bSignificantly different between Rest and Ac-Ex groups, $P < 0.05$. ^cSignificantly different between Ac-Ex groups, $P < 0.05$; ^{cc} $P < 0.01$.

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 glycogen 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 glycogen 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-

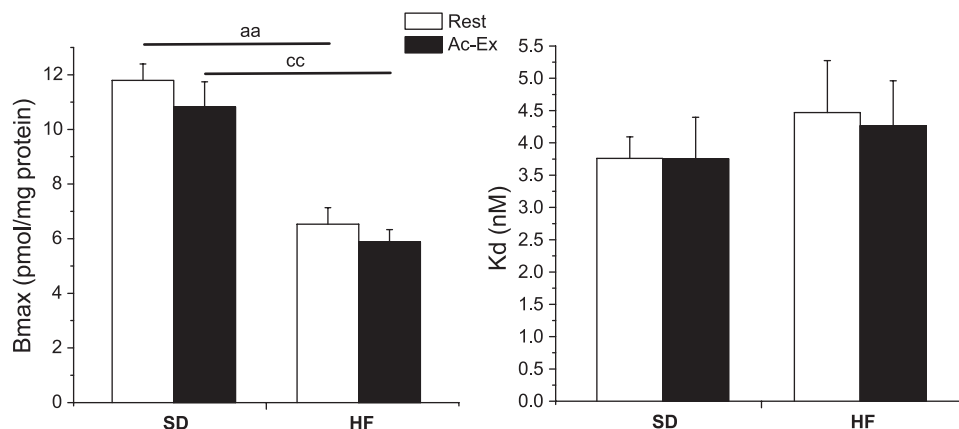


Fig. 3. Glucagon receptor density (B_{max}) and affinity (K_d) in Rest and Ac-Ex rats after 2 wk of an SD or HF diet. Values are means \pm SE; $n = 8-10$ rats/group. ^{aa}Significantly different between SD- and HF-fed groups, $P < 0.01$; ^{cc}significantly different between Ac-Ex groups, $P < 0.01$.

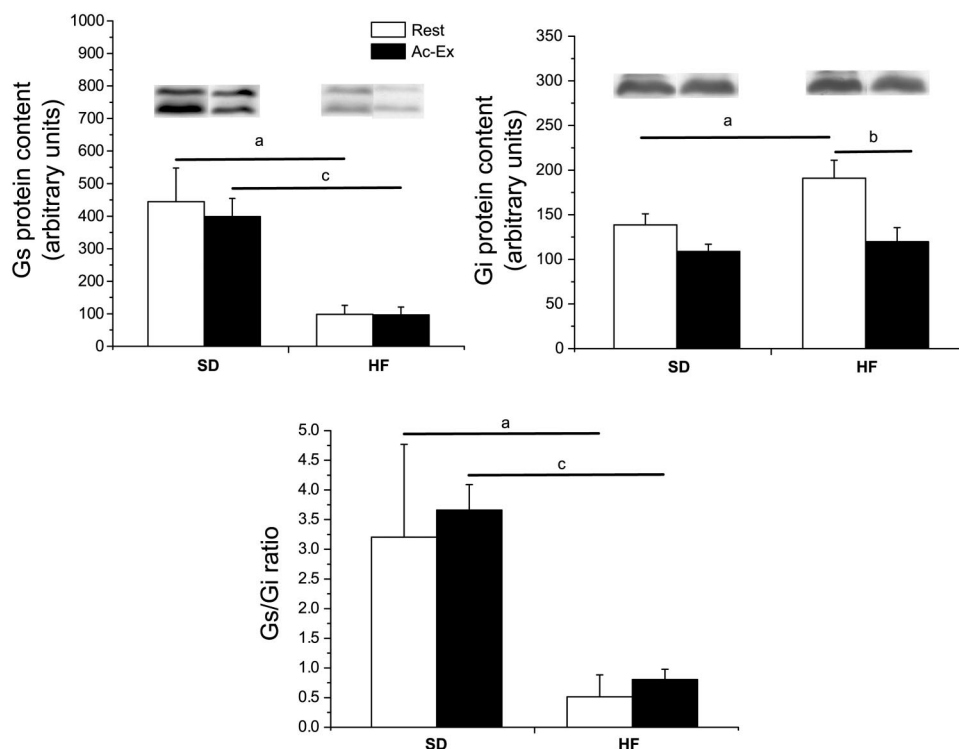


Fig. 4. Stimulatory and inhibitory α -subunits of hepatic G proteins ($G_s\alpha$ and $G_i\alpha_2$) and the $G_s\alpha/G_i\alpha_2$ ratio in Rest and Ac-Ex rats after 2 wk of an SD or HF diet. Values are means \pm SE; $n = 8-10$ rats/group. ^aSignificantly different between the SD- and HF-fed groups, $P < 0.05$; ^bSignificantly different between Rest and Ac-Ex groups, $P < 0.05$. ^cSignificantly different between Ac-Ex groups, $P < 0.05$.

tions in the HF/Ac-Ex group compared with its SD-fed counterpart.

In agreement with our hypotheses, glucagon receptor density (B_{max}) was significantly ($P < 0.01$) reduced with the feeding of an HF diet (Fig. 3). An $\sim 45\%$ decrease in B_{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 $G_s\alpha$ 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 $G_s\alpha$ content. There was an $\sim 27\%$ ($P < 0.05$) increase in $G_i\alpha_2$ content with the feeding of the HF diet in the resting state. The acute exercise bout significantly ($P < 0.05$) reduced $G_i\alpha_2$ content under the HF diet (Fig. 4). Even though $G_i\alpha_2$ content was increased in the HF/Rest group, the $G_s\alpha/G_i\alpha_2$ ratio was $\sim 80\%$ lower in the HF- compared with the SD-fed groups (Fig. 4). Acute exercise had no effect on the $G_s\alpha/G_i\alpha_2$ ratio.

Figure 5 shows the association between the level of hepatic triglycerides and the B_{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_{max}), accompanied by a 75% lower plasma membrane level of $G_s\alpha$ protein

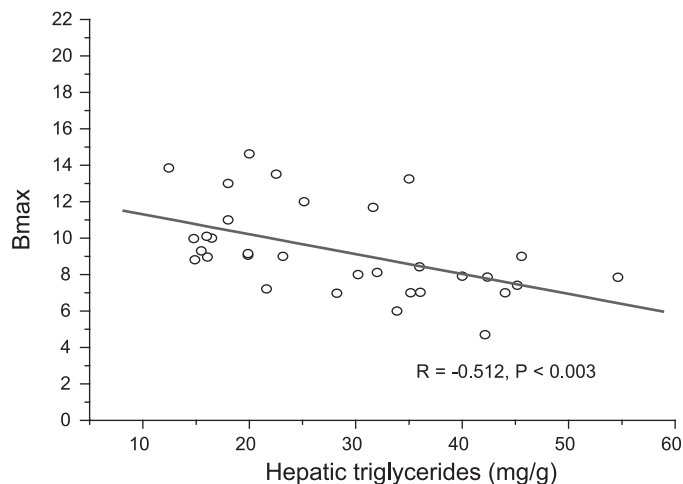


Fig. 5. Relationship between glucagon receptor density (B_{max}) and liver triglyceride concentrations ($n = 32$, $P < 0.003$) for all rats in all dietary and rest-exercise conditions.

content, and a lower $G_s\alpha/G_i\alpha_2$ 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 $G_s\alpha/G_i\alpha_2$ 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 $G_s\alpha$ 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 Légaré 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 $G_s\alpha$ 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 α -subunit of stimulatory G protein than those from their lean littermates (2, 15). However, the present HF diet-induced increase in $G_i\alpha_2$ content (27%) has not been found in other studies. Begin-Heick (2) and Gettys et al. (15) observed a significant decrease in $G_i\alpha_2$ protein content in liver plasma membranes of *db/db* and *ob/ob* mice. Nevertheless, the $G_s\alpha/G_i\alpha_2$ ratio was largely decreased by the present HF diet regimen. Podolin et al. (32) reported a reduced protein expression of $G_s\alpha$ and $G_s\alpha/G_i\alpha_2$ 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 $G_s\alpha/G_i\alpha_2$ activity observed in these studies was influenced by the liver lipid content. On the whole, the present decrease in protein expression of $G_s\alpha$ and in the $G_s\alpha/G_i\alpha_2$ 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 $G_s\alpha$ protein content and $G_s\alpha/G_i\alpha_2$ 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_i 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, $G_s\alpha$ protein content, and $G_s\alpha/G_i\alpha_2$ 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

This work was supported by grants from Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. Abrahamsen N, Lundgren K, and Nishimura E. Regulation of glucagon receptor mRNA in cultured primary rat hepatocytes by glucose and cAMP. *J Biol Chem* 270: 15853–15857, 1995.

2. **Begin-Heick N.** Liver beta-adrenergic receptors, G proteins, and adenylyl cyclase activity in obesity-diabetes syndromes. *Am J Physiol Cell Physiol* 266: C1664–C1672, 1994.
3. **Bhathena SJ, Voyles NR, Smith S, and Recant L.** Decreased glucagon receptors in diabetic rat hepatocytes. Evidence for regulation of glucagon receptors by hyperglucagonemia. *J Clin Invest* 61: 1488–1497, 1978.
4. **Buettner R, Ottlinger I, Schölmerich J, and Bollheimer C.** Preserved direct hepatic insulin action in rats with diet-induced hepatic steatosis. *Am J Physiol Endocrinol Metab* 286: E828–E833, 2004.
5. **Burcelin R, Mrejen C, Decaux JF, De Mouzon SH, Girard J, and Charron MJ.** In vivo and in vitro regulation of hepatic glucagon receptor mRNA concentration by glucose metabolism. *J Biol Chem* 273: 8088–8093, 1998.
6. **Caro JF, Raju MS, Caro M, Lynch CJ, Poulos J, Exton JH, and Thakkar JK.** Guanine nucleotide binding regulatory proteins in liver from obese humans with and without type II diabetes: evidence for altered “cross-talk” between the insulin receptor and Gi-proteins. *J Cell Biochem* 54: 309–319, 1994.
7. **Charbonneau A, Couturier K, Gauthier MS, and Lavoie JM.** Evidence of hepatic glucagon resistance associated with hepatic steatosis: reversal effect of training. *Int J Sports Med* doi:10.1055/s-2004-821225.
8. **DeBburman SK, Ptasienski J, Benovic JL, and Hosey MM.** G protein-coupled receptor kinase GRK2 is a phospholipid-dependent enzyme that can be conditionally activated by G protein betagamma subunits. *J Biol Chem* 271: 22552–22562, 1996.
9. **Dighe RR, Rojas FJ, Birnbaumer L, and Garber AJ.** Glucagon-stimulable adenylyl cyclase in rat liver. The impact of streptozotocin-induced diabetes mellitus. *J Clin Invest* 73: 1013–10123, 1984.
10. **Drouin R, Lavoie C, Bourque J, Ducros F, Poisson D, and Chiasson JL.** Increased hepatic glucose production response to glucagon in trained subjects. *Am J Physiol Endocrinol Metab* 274: E23–E28, 1998.
11. **Drouin R, Robert G, Milot M, Massicotte D, Péronnet F, and Lavoie C.** Swim training increases glucose output from liver perfused in situ with glucagon in fed and fasted rats. *Metabolism* 53: 1027–1031, 2004.
12. **Frandsen EK, Thim L, Moody AJ, and Markussen J.** Structure-function relationships in glucagon. Re-evaluation of glucagon-(1–21). *J Biol Chem* 260: 7581–7584, 1985.
13. **Gauthier MS, Couturier K, Charbonneau A, and Lavoie JM.** Effects of introducing physical training in the course of a 16-wk high-fat diet regimen on hepatic steatosis, adipose tissue fat accumulation, and plasma lipid profile. *Int J Obes* 28: 1064–1071, 2004.
14. **Gauthier MS, Couturier K, Latour JG, and Lavoie JM.** Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol* 94: 2127–2134, 2003.
15. **Gettys TW, Ramkumar V, Surwit RS, and Taylor IL.** Tissue-specific alterations in G protein expression in genetic vs. diet-induced models of noninsulin-dependent diabetes mellitus in the mouse. *Metabolism* 44: 771–778, 1995.
16. **Hennes MM, Shrago E, and Kissebah AH.** Receptor and postreceptor effects of free fatty acids on hepatocyte insulin dynamics. *Int J Obes* 14: 829–830, 1990.
17. **Hammond HK, Roth DA, McKirnan MD, and Ping P.** Regional myocardial down-regulation of the inhibitory GTP-binding protein (Gi alpha 2) and beta-adrenergic receptors in a porcine model for chronic episodic myocardial ischemia. *J Clin Invest* 92: 2644–2652, 1993.
18. **Jiang G and Zhang BB.** Glucagon and regulation of glucose metabolism. *Am J Physiol Endocrinol Metab* 284: E671–E678, 2003.
19. **Kahn CR, Neville DM, and Roth J.** Insulin-receptor interaction in the obese-hyperglycemic mouse. A model of insulin resistance. *J Biol Chem* 248: 244–250, 1973.
20. **Keller U, Sonnenberg GE, Burckhardt D, and Perruchoud A.** Evidence for an augmented glucagon dependence of hepatic glucose production in cirrhosis of the liver. *J Clin Endocrinol Metab* 54: 961–968, 1982.
21. **Krones A, Kietzmann T, and Jungermann K.** Periportal localization of glucagon receptor mRNA in rat liver and regulation of its expression by glucose and oxygen in hepatocyte cultures. *FEBS Lett* 421: 136–140, 1998.
22. **Lam TK, Yoshii H, Haber CA, Bogdanovic E, Lam L, Fantus IG, and Giacca A.** Free fatty acid-induced hepatic insulin resistance: a potential role for protein kinase C- δ . *Am J Physiol Endocrinol Metab* 283: E682–E691, 2002.
23. **Lefkowitz RJ.** G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. *J Biol Chem* 273: 18677–18680, 1998.
24. **Lefkowitz RJ, Pitcher J, Krueger K, and Daaka Y.** Mechanisms of beta-adrenergic receptor desensitization and resensitization. *Adv Pharmacol* 42: 416–420, 1998.
25. **Légaré A, Drouin R, Milot M, Massicotte D, Péronnet F, Massicotte G, and Lavoie C.** Increased density of glucagon receptors in liver from endurance-trained rats. *Am J Physiol Endocrinol Metab* 280: E193–E196, 2001.
26. **Lewis GF, Carpentier A, Adeli K, and Giacca A.** Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23: 201–229, 2002.
27. **Lo S, Russell JC, and Taylor AW.** Determination of glycogen in small tissue samples. *J Appl Physiol* 28: 234–236, 1970.
28. **Matas Bonjorn V, Latour MG, Bélanger P, and Lavoie JM.** Influence of prior exercise and liver glycogen content on the sensitivity of the liver to glucagon. *J Appl Physiol* 92: 188–194, 2002.
29. **Murawski U, Kriesten K, and Egge H.** Age related changes of lipid fractions and total fatty acids in liver lipids and heart lipids of female and male rats aged 37–1200 days (liver) and 331–1200 days (heart). *Comp Biochem Physiol B* 96: 271–289, 1990.
30. **Orskov L, Alberti KG, Mengel A, Moller N, Pedersen O, Rasmussen O, Seefeldt T, and Schmitz O.** Decreased hepatic glucagon responses in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 34: 521–526, 1991.
31. **Pitcher JA, Fredericks ZL, Stone WC, Premont RT, Stoffel RH, Koch WJ, and Lefkowitz RJ.** Phosphatidylinositol 4,5-bisphosphate (PIP₂)-enhanced G protein-coupled receptor kinase (GRK) activity. Location, structure, and regulation of the PIP₂ binding site distinguishes the GRK subfamilies. *J Biol Chem* 271: 24907–24913, 1996.
32. **Podolin DA, Wills BK, Wood IO, Lopez M, Mazzeo RS, and Roth DA.** Attenuation of age-related declines in glucagon-mediated signal transduction in rat liver by exercise training. *Am J Physiol Endocrinol Metab* 281: E516–E523, 2001.
33. **Pohl SL.** The glucagon receptor and its relationship to adenylate cyclase. *Fed Proc* 36: 2115–2118, 1977.
34. **Portois L, Maget B, Tastenoy M, Perret J, and Svoboda M.** Identification of a glucose response element in the promoter of the rat glucagon receptor gene. *J Biol Chem* 274: 8181–8190, 1999.
35. **Rao RH.** Adaptations in glucose homeostasis during chronic nutritional deprivation in rats: hepatic resistance to both insulin and glucagon. *Metabolism* 44: 817–824, 1995.
36. **Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Belfroy D, Romanelli AJ, and Shulman GI.** Mechanism of hepatic insulin resistance in nonalcoholic fatty liver disease. *J Biol Chem* 279: 32345–32353, 2004.
37. **Strackowski M, Kowalska I, Dzienis-Strackowska S, Kinalski M, Gorski J, and Kinalska I.** The effect of exercise training on glucose tolerance and skeletal muscle triacylglycerol content in rats fed with a high-fat diet. *Diabetes Metab* 27: 19–23, 2001.
38. **Svoboda M, Portois L, and Malaisse WJ.** Glucose regulation of the expression of the glucagon receptor gene. *Mol Genet Metab* 68: 258–267, 1999.