Effects of high-fat diet and exercise training on intracellular glucose metabolism in rats

CHUL-HEE KIM,1 J ANG H. YOUN,2 J OONG-YEOL PARK,3 SUNG K. HONG,3
KYONG S. PARK,4 SUNG W. PARK,5 KYO I. SUH,1 AND KI-UP LEE3

1Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul 140-743;
2Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-744; and
3Hallym University College of Medicine, Anyang 431-070, Korea;
and 4Department of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, California 90089-9142

Kim, Chul-Hee, J ang H. Youn, J oong-Yeol Park, Sung K. Hong, Kyong S. Park, Sung W. Park, Kw o I. Suh, and Ki-Up Lee. Effects of high-fat diet and exercise training on intracellular glucose metabolism in rats. Am J Physiol Endocrinol Metab 278: E977–E984, 2000.—We examined the effects of high-fat diet (HFD) and exercise training on insulin-stimulated whole body glucose fluxes and several key steps of glucose metabolism in skeletal muscle. Rats were maintained for 3 wk on either low-fat (LFD) or high-fat diet with or without exercise training (swimming for 3 h per day). After the 3-wk diet/exercise treatments, animals underwent hyperinsulinenic euglycemic clamp experiments for measurements of insulin-stimulated whole body glucose fluxes. In addition, muscle samples were taken at the end of the clamps for measurements of glucose 6-phosphate (G-6-P) and GLUT-4 protein contents, hexokinase, and glycogen synthase (GS) activities. Insulin-stimulated glucose uptake was decreased by HFD and increased by exercise training (P < 0.01 for both). The opposite effects of HFD and exercise training on insulin-stimulated glucose uptake were associated with similar increases in muscle G-6-P levels (P < 0.05 for both). However, the increase in G-6-P level was accompanied by decreased GS activity without changes in GLUT-4 protein content and hexokinase activities in the HFD group. In contrast, the increase in G-6-P level in the exercise-trained group was accompanied by increased GLUT-4 protein content and hexokinase II (cytosolic) and GS activities. These results suggest that HFD and exercise training affect insulin sensitivity by acting predominantly on different steps of intracellular glucose metabolism. High-fat feeding appears to induce insulin resistance by affecting predominantly steps distal to G-6-P (e.g., glycolysis and glycogen synthesis). Exercise training affected multiple steps of glucose metabolism both proximal and distal to G-6-P. However, increased muscle G-6-P levels in the face of increased glucose metabolic fluxes suggest that the effect of exercise training is quantitatively more prominent on the steps proximal to G-6-P (i.e., glucose transport and phosphorylation).

glucose metabolic fluxes; glucose 6-phosphate; glucose transporter; hexokinase; glycogen synthase

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.
exercise training were attributed to a primary change in the initial steps of glucose metabolism (i.e., glucose transport and/or phosphorylation). This concept is supported by the findings that exercise training increases GLUT-4 content (33, 41), glucose transport (41), and hexokinase activity in skeletal muscle (11, 27). However, exercise training also has profound stimulatory effects on key enzymes of glucose metabolism in skeletal muscle, such as GS (15, 39), and it is unclear whether exercise training improves insulin action mainly by enhancing the glucose transport/phosphorylation step or by stimulating steps of glucose metabolism distal to glucose 6-phosphate (G-6-P).

This study was undertaken to identify the predominant sites of regulation of insulin action by HFD and exercise training. G-6-P is an intermediate in glucose metabolism, and its concentration may reflect the relative activities of its proximal (glucose transport and metabolism, and its concentration may reflect the exercise training. G-6-

close fluxes and on muscle G-6-
lism distal to glucose 6-phosphate (G-6-P).

Experimental protocols. Food was removed from the cages at 7 AM. To estimate basal whole body glucose turnover, $\varphi$-[3-3H]glucose (New England Nuclear, Boston, MA) was infused at a rate of 0.06 $\mu$Ci/min for 3 h (basal period) starting at 10 AM. Two blood samples were collected during the last 20 min of the basal period for measurements of plasma glucose, free fatty acids (FFA), and $[3H]$glucose concentrations. The basal period was followed by a 2-h hyperinsulinemic euglycemic clamp (clamp period) in which porcine insulin (Novo-Nordisk, Gentofte, Denmark) was continuously infused at a rate of 72 pmol·kg$^{-1}$·min$^{-1}$. Blood samples (40 µl) were collected at 10-min intervals for the immediate measurement of plasma glucose, and 25% dextrose was infused at variable rates to maintain plasma glucose at basal concentrations ($\sim$7.6 mM). To estimate whole body insulin-stimulated glucose fluxes during clamps, $\varphi$-[3-3H]glucose was infused at a rate of 0.3 $\mu$Ci/min throughout the clamp. Blood samples (120 µl) were taken for the determination of plasma $[3H]$glucose and $[3H]$O2 concentrations at 10-min intervals during the final 40 min of the clamps. Additional blood samples (100 µl) were obtained at 30, 60, 90, and 120 min for the determination of plasma insulin and FFA concentrations. At the end of the clamp, rats were anesthetized with an intravenous injection of pentobarbital, and gastrocnemius muscles were frozen in situ by aluminum tongs precooled in liquid nitrogen. The frozen muscle samples were stored at $-70^\circ$C for later analysis.

Analysis of plasma samples. Plasma glucose was measured by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Palo Alto, CA). Plasma FFA was measured by an enzymatic assay using a kit from Eiken Chemical (Tokyo, Japan). Plasma insulin was measured by radioimmunoassay with kits for rat (basal insulin; Linco, St. Charles, MO) and human insulin (damp insulin; Dainabot, Tokyo, Japan). Plasma $[3H]$glucose radioactivity was measured in duplicate by deproteinizing plasma samples with saturated $\text{Ba(OH)}_2$ and 5.5% $\text{ZnSO}_4$ drying to eliminate tritiated water, and counting for $[3H]$ in a liquid scintillation spectrophotometer (Beckman Instruments). The plasma concentration of $[3H]$-labeled water was determined by the difference between $[3H]$ counts with and without drying.

Muscle G-6-P level. Skeletal muscle G-6-P level was determined by an enzymatic assay as described by Michal (26). Because muscle G-6-P concentration may be sensitive to plasma glucose, glucose infusion was continued during the muscle sampling procedures to prevent any significant perturbation of plasma glucose concentration. Care was also taken to prevent G-6-P concentration from rising because of glycogenolysis during the procedures. Frozen muscles were crushed...
in liquid nitrogen and homogenized with 6% perchloric acid at 0°C.

Muscle GS activity. GS activity was measured according to the method of Golden et al. (9) with minor modifications. We used the superficial part of gastrocnemius muscle, which mainly consists of white muscle fibers, for the determination of GS activity because we had previously observed that superficial parts of gastrocnemius muscles have higher GS activities than deep parts of the muscle (31). Total GS activity, reflecting the amount of GS protein, was determined at a G-6-P concentration of 10 mM. The proportion of the active form of the enzyme was determined by the ratio of GS activity to the total activity in the absence of G-6-P (GSI, G-6-P independent) or at 0.1 mM G-6-P (fractional velocity).

The radioactivity was then determined on a liquid scintillation counter. The amount of 3H in muscle glycogen was expressed as disintegrations per minute per gram of tissue wet weight.

GLUT-4 protein content. Fifty milligrams of muscle were homogenized using a polytron at 4°C in a buffer containing 30 mM HEPES, 0.6 M KCl, 5 µg/ml leupeptin, 5 µg/ml aprotonin, 1 µg/ml pepstatin, and 400 µM phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 1,000 g for 15 min at 4°C. The supernatants were centrifuged at 388,000 g for 100 min at 4°C. The pellets (membrane fraction) were resuspended in 20 mM Tris, 255 mM sucrose, and 1 mM EDTA (pH 7.4), and protein concentrations were determined. The membrane preparations were diluted 1:1 in 2× Laemmli's buffer without β-mercaptoethanol. Proteins were separated on 10% SDS-PAGE gels and then transferred to nitrocellulose membranes. Polyclonal antiserum specific for GLUT-4 (RaIRGT, East Acres Biologicals, Cambridge, MA) and anti-rabbit IgG conjugated with horseradish peroxidase (Amersham, Arlington Heights, IL) were used. Immune complexes were detected using an enhanced chemiluminescence kit (Amersham). Quantitation of GLUT-4 immunoreactivity was performed by densitometric scanning.

Hexokinase activity. Cytosolic and crude mitochondrial fractions were prepared by ultracentrifugation of muscle homogenates at 20,000 g for 15 min at 4°C. Hexokinase activity was determined by coupling G-6-P production to NADPH formation via the glucose-6-phosphate dehydrogenase reaction (24). One aliquot of the sample was assayed to determine total hexokinase activity (hexokinase I and hexokinase II), and another aliquot was heated for 1 h at 45°C and then assayed for heat-stable hexokinase I activity (10). Hexokinase II activity was determined by subtracting hexokinase I from total hexokinase activity. Enzyme activities were expressed as nanomoles per minute per milligram protein.

Radioisotopic determination of glucose fluxes. Rates of total glucose appearance and whole body glucose uptake were determined as the ratio of the [3H]glucose infusion rate (dpm/min) to the specific activity of plasma glucose (dpm/µmol) during the final 40 min of the clamp (42). Whole body glycolysis was calculated from the rate of increase in plasma 3H2O concentration during the final 40 min of the clamp, as previously described (37). The rate of increase in plasma 3H2O was determined by linear regression of the measurements at 10-min intervals during the final 40 min of the clamp. Whole body glycogen synthesis was estimated by subtracting whole body glycolysis from whole body glucose uptake, with the assumption that glycolysis and glycogen synthesis account for the majority of insulin-stimulated glucose uptake (37).

Statistical analysis. Data are presented as means ± SE. The significance of the effects of HFD and exercise training was assessed by two-way ANOVA.

RESULTS

Basal metabolic parameters. Table 1 shows average food and caloric intakes and changes in body weight during the 3-wk period of diet/exercise treatment. Food intake (by weight) was significantly lower in the HFD than in the LFD groups (P < 0.05). However, there was no significant difference in daily caloric intake among the four groups. The gain in body weight during the treatment period was slightly lower in the exercise than in the sedentary groups, but the differences were not statistically significant (P > 0.05). On the experimental day (i.e., after the 3-wk treatment period), basal plasma glucose and insulin concentrations were similar in all four groups (Fig. 1; time 0). Plasma FFA concentrations were higher in the HFD than in the LFD groups, but these differences were not statistically significant (P > 0.05).

Hyperinsulinemic euglycemic clamps. During the clamps, plasma insulin concentrations were raised to similar levels (~1,200 pM) in all groups, and plasma glucose levels were maintained at basal (~7.6 mM) levels (Fig. 1). Plasma FFA levels decreased similarly in all groups. Glucose infusion rates required to maintain euglycemia were significantly lower in the HFD groups than in the LFD groups (P < 0.01). Exercise training significantly increased the rates of glucose infusion required during the clamps (P < 0.01).

Insulin-stimulated whole body glucose fluxes. Similar to the changes in glucose infusion rates, insulin-stimulated glucose uptake (during the final 40 min of the clamp) was increased by exercise training (P < 0.01) and decreased by HFD (P < 0.01; Fig. 2A). Effects of exercise training and HFD on insulin-stimulated glycolysis and glycogen synthesis were similar to those on insulin-stimulated glucose uptake (Fig. 2, B and C). There was no significant interaction between the effects of diet and exercise treatment on any of these fluxes.

Accumulation of [3H]glycogen and GS activity. Incorporation of [3H]glucose into muscle glycogen was significantly increased by exercise training (P < 0.01; Fig. 3A) but decreased by HFD (P < 0.05). Total GS activity was not statistically different between the four groups (P > 0.05).

Table 1. Food and caloric intakes and changes in body weight during 3 wk of diet and/or exercise treatment

<table>
<thead>
<tr>
<th></th>
<th>LFD-S</th>
<th>LFD-Ex</th>
<th>HFD-S</th>
<th>HFD-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average food intake, g/day</td>
<td>34.1 ± 2.6</td>
<td>34.5 ± 2.0</td>
<td>24.2 ± 1.8*</td>
<td>25.1 ± 1.7*</td>
</tr>
<tr>
<td>Average calorie intake, kJ/day</td>
<td>514 ± 38</td>
<td>519 ± 29</td>
<td>506 ± 37</td>
<td>522 ± 35</td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>258 ± 8</td>
<td>255 ± 7</td>
<td>261 ± 10</td>
<td>259 ± 7</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>340 ± 11</td>
<td>325 ± 10</td>
<td>367 ± 13</td>
<td>347 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. LFD-S, low-fat diet sedentary group; LFD-Ex, LFD exercise group; HFD-S, high-fat diet sedentary group; HFD-Ex, HFD exercise group. *P < 0.05 vs. LFD groups.
significantly increased by exercise training ($P < 0.01$) but unchanged by HFD (Fig. 3B). GSI ratio (Fig. 3C) and the fractional velocity (data not shown) of GS were also significantly increased by exercise training ($P < 0.05$) but decreased by HFD ($P < 0.01$).

Muscle G-6-P concentration. Muscle G-6-P content at the end of the clamps was significantly increased by both exercise and HFD ($P < 0.05$ for each; Fig. 3D). The effects of exercise training and HFD on muscle G-6-P content were additive, and the interaction between the effects of the two treatments was not significant.

GLUT-4 protein content and hexokinase activity. Total muscle content of GLUT-4 protein was similar in the LFD and HFD groups. In contrast, exercise training significantly increased GLUT-4 protein content ($P < 0.05$; Fig. 4). Exercise training also significantly increased cytosolic hexokinase II activity ($P < 0.01$; Fig. 5) without affecting the activities of hexokinase I or of hexokinase II in the mitochondrial fraction (data not shown). HFD did not affect muscle hexokinase activities. There was no significant interaction between the effects of diet and exercise treatment on GLUT-4 content or hexokinase activities.

DISCUSSION

The present study in rats confirmed that high-fat feeding induces insulin resistance (16, 19, 20, 43, 47) and exercise training improves insulin action in vivo (18, 21, 33, 41). Of note in our study is that the opposite effects of HFD and exercise training on insulin-stimulated glucose uptake were associated with similar increases in muscle G-6-P level. Muscle G-6-P levels
may reflect the relative activities of G-6-P production (via glucose transport and phosphorylation) and utilization (via glycogen synthesis and glycolysis). Therefore, increased muscle G-6-P levels may indicate increased activities of glucose transport/phosphorylation and/or decreased activities of glucose metabolism distal to G-6-P. In the HFD-fed rats, increased G-6-P levels were associated with reduced glucose metabolic fluxes, indicating an impairment of glucose metabolism distal to G-6-P. Indeed, we found that insulin-stimulated muscle GS activity (active form, but not total) was profoundly reduced by HFD. Thus high-fat feeding appears to induce insulin resistance by causing a predominant impairment at steps of glucose metabolism distal to G-6-P, which may occur as a consequence of increased fat oxidation (see the next paragraph). In the exercise-trained groups, significant improvements were observed in many aspects of glucose metabolism, including whole body glycolysis and glycogen synthesis, muscle GLUT-4 protein content, hexokinase activity, and GS activity. However, increased muscle G-6-P levels in the face of increased glucose metabolic fluxes indicate that the effect of exercise training is quantitatively more prominent on the steps proximal than distal to G-6-P.

Previous studies have well documented impaired insulin stimulation of glycolysis in animals maintained on HFD (20, 21). The inhibitory effect of HFD on insulin-stimulated glycolysis is likely due to increased lipid oxidation (the glucose-fatty acid cycle) (19, 34). Increased fatty acid oxidation may increase the acetyl CoA-to-CoASH ratio (8) and the concentration of citrate (35) in cardiac and/or skeletal muscle. Acetyl-CoA is known to inhibit pyruvate dehydrogenase complex, the rate-limiting step for glucose oxidation, and citrate is a potent inhibitor of phosphofructokinase (28), a key enzyme for glycolysis. Kim et al. (19) recently provided evidence that phosphofructokinase activity was inhibited in skeletal muscle of high-fat-fed rats.

In addition to the impairment of insulin stimulation of glycolysis, we found that insulin-stimulated whole body glycogen synthesis was significantly decreased after 3 wk of HFD feeding. This decrease was accompanied by reduced GS activity (of the active form, but not total) and reduced accumulation of [3H]glycogen in skeletal muscle, suggesting that reduced activity of the active (dephosphorylated) form of GS was responsible for decreased whole body glycogen synthesis. The mechanism by which HFD reduces insulin’s ability to activate skeletal muscle GS is not established. One possibl...
ity is that GS is inhibited (or phosphorylated) in HFD-fed rats by fatty acid metabolites, such as long-chain acyl-CoA (29). Alternatively, increased substrate flux through the hexosamine biosynthesis pathway may decrease insulin sensitivity of GS activation and glycogen synthesis (14). Whatever the mechanism, the significance of the present study is the demonstration of a defect in HFD-fed rats similar to that observed in human obesity or type 2 diabetes. However, reduced insulin-stimulated GS activity may not be the primary event leading to insulin resistance in HFD-fed rats. Kim et al. (19) recently reported that insulin-stimulated glycogen synthesis was increased (rather than decreased, as in the present study) during the initial few days of high-fat feeding and was subsequently decreased to control values by 2 wk of high-fat feeding. Taken together with the present findings, these data suggest that the defect in insulin stimulation of GS may take substantial time (2–3 wk) to develop. Kim et al. also showed that insulin-stimulated glycolysis was significantly decreased within 40–50% decreases in insulin stimulation of glucose transport without altering GLUT-4 content (12, 13), presumably because of an impairment of insulin action on GLUT-4 translocation (47). Although insulin-stimulated glucose transport or GLUT-4 translocation was not directly assessed in the present study, significant decreases in insulin-stimulated glucose uptake in the HFD-fed rats are consistent with an impairment of insulin action on glucose transport. However, because decreased insulin-stimulated glucose uptake was associated with increased muscle G-6-P levels in HFD-fed rats, it is conceivable that glucose metabolic steps distal to G-6-P were more profoundly impaired than glucose transport or phosphorylation steps in these rats.

Recent human studies (5, 17) have suggested that mechanisms other than the glucose-fatty acid cycle are responsible for insulin resistance in type 2 diabetes or visceral obesity, on the basis of the finding that skeletal muscle utilization of FFA was decreased, rather than increased, in these subjects. Diminished fat oxidation capacity may increase cytosolic long-chain fatty acyl-CoA (5, 17), which has been shown to impair insulin action on glucose transport by altering insulin signaling events (6, 47). Therefore, we cannot exclude the possibility that this mechanism, rather than the glucose-fatty acid cycle, was the major mechanism responsible for the development of insulin resistance in HFD-fed rats. However, such a mechanism (i.e., a primary defect in glucose transport) may be inconsistent with the present finding that the predominant site of impairment in glucose metabolism was distal to G-6-P rather than glucose transport.

Exercise training increased total GLUT-4 protein content and hexokinase II (cytosolic) activity in skeletal muscle. These results are in agreement with previous studies (11, 27, 33, 41) and support the concept that exercise training improves insulin action on glucose utilization by increasing glucose transport and/or phosphorylation. Also in agreement with previous studies (15, 39) were the findings that exercise training enhanced insulin stimulation of intracellular glucose...
metabolism and GS activity. Thus exercise training appears to enhance both glucose entry into cells and its metabolism inside cells. However, the influence of exercise training appears to enhance both glucose entry into cells and its metabolism and GS activity. Thus exercise training by improving insulin sensitivity by mainly increasing the exercise training appears to be quantitatively more metabolism inside cells. However, the influence of exercise training appears to enhance both glucose entry into cells and its metabolism and GS activity. Thus exercise training by impairing glucose metabolism predominantly at steps distal to G-6-P, whereas exercise training may improve insulin sensitivity by mainly increasing the activity of glucose transport and/or phosphorylation.

This study was supported by grants from the Asan Institute for Life Sciences (99–006), the Korean Association of Internal Medicine, and BIOTECH CH 2000 of the National R&D Program (98-N1–02–04-A-07), Ministry of Science and Technology, Seoul, Korea.

Address for reprint requests and other correspondence: K.-U. Lee, Dept. of Internal Medicine, Univ. of Ulsan College of Medicine, 388-1 Poong-Nap Dong, Songpa Ku, Seoul 138–736, Korea (E-mail: kulee@uams.seoul.kr).

Received 10 August 1999; accepted in final form 5 January 2000.

REFERENCES

30. Olefsky J M, Koltermann OG, and Scarlett J A. Insulin action and resistance in obesity and noninsulin-dependent type II


