Interactive Effect of Exercise Training and Growth Hormone Administration on Glucose Tolerance and Muscle GLUT4 Protein Expression in Rats

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Key Words
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Abstract
The insulin-resistance effect of growth hormone (GH) administration has been frequently reported. The present study investigated the effect of GH administration on glucose tolerance and muscle GLUT4 protein expression in exercise-trained and untrained rats. Forty-eight rats were weight-matched and assigned to the following 4 groups: control, GH, exercise training, and exercise training + GH groups. After 2 weeks of GH injections (65 μg/kg/day) and exercise training, the glucose tolerance and insulin response were measured in these rats. The GLUT4 protein level, glycogen storage, and citrate synthase activity were determined in red gastrocnemius and plantaris muscles. Daily GH administration elevated the curves of the oral glucose tolerance test and insulin response compared with those of saline-injected control rats. Furthermore, exercise training completely eliminated this GH-induced insulin resistance as determined 18 h after the last bout of exercise training. Additionally, exercise training significantly increased muscle glycogen storage and GLUT4 protein levels. GH administration did not affect the GLUT4 protein and glycogen storage increases induced by exercise training, but the citrate synthase activity in the plantaris muscle was further elevated by GH administration to a level above that induced by training. In conclusion, this is the first study that demonstrates that regular exercise training prevents GH-induced insulin-resistance side effect in rats.

Introduction
Under postprandial conditions, skeletal muscle is the most important tissue for blood glucose disposal in the body [2, 8]. Therefore, changes in muscle glucose transport properties will have consequent ramifications on the entire body glycemic control. Due to the hydrophilic property of the glucose molecule, the glucose transport process requires specific 12-pass transmembrane transporters to facilitate glucose diffusion across plasma membranes [20]. In skeletal muscle, insulin-stimulated glucose transport is mediated by the GLUT4 protein, which is
familiarize the use of recombinant human GH as a therapeutic modality has greatly increased for such conditions as aging, short stature, protein wasting after surgery, burns, and trauma [10]. It has now expanded beyond the use as a replacement for GH deficiency. However, many early studies showed that acute or chronic GH administration can cause insulin resistance and glucose intolerance in humans and animals [1, 11]. The underlying mechanism of how GH regulates glycemic control remains unclear. Early evidence indicated that GH-induced insulin resistance was associated with reduced insulin-stimulated glucose disposal and glycogen storage in skeletal muscles [11]. Although it was reported that high-fructose- and high-fat-diet-induced insulin resistance could be overcome by regular exercise training [16, 21], current investigations on exercise-training effect on GH-induced insulin resistance are inadequate. Therefore, the purpose of the present study was to determine the interactive effect of GH administration and exercise training on glucose tolerance and muscle glycogen storage. Muscle GLUT4 protein levels and citrate synthase activity, both of which are involved with glucose metabolism, were also studied.

**Methods**

**Housing and Care of the Animals**

Forty-eight male Sprague-Dawley rats from the National Animal Laboratory of the NSC (National Science Council, Taipei, Taiwan, ROC) weighing 180 g each were housed 3 per cage and were provided normal rat chow (PMI Nutrition International, Brentwood, Mo., USA) and water ad libitum. The temperature of the animal room was maintained at 21 °C, with a 12-hour light-dark cycle. After 1 week of

one of a family of glucose transporters found in various tissues, being especially enriched in skeletal muscle [20]. Evidence has shown that the total amount of GLUT4 protein and the amount of this protein translocated to the sarcolemma determine the insulin responsiveness of muscle glucose uptake [19]. It is now widely accepted that this protein molecule plays a key role in whole-body insulin sensitivity and glucose tolerance [14, 24, 28]. In fact, several studies have demonstrated a correlation between the total amount of GLUT4 protein in skeletal muscle and whole-body glucose disposal [7, 19]. Early studies frequently demonstrated that exercise training is one effective intervention for increasing muscle GLUT4 protein expression [17, 18]. In addition, the elevation in GLUT4 protein content with exercise training typically occurs in parallel with improvements in glucose tolerance and insulin sensitivity [7, 14].

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**Oral Glucose Tolerance Test (OGTT)**

At the end of 2-week exercise training and GH administration, an OGTT was performed 18 h after the last bout of exercise, according to Holloszy et al. [12]. This recovery period included a 12-hour fast prior to the glucose infusion for OGTT. Blood samples were withdrawn from the tail at 0 (fasted sample), 15, and 45 min after the oral glucose load (1 g/kg BW) for blood glucose and serum insulin measurements, according to the procedure given in Cortez et al. [7]. A glucose analyzer (Lifescan, Milpitas, Calif., USA) was used for glucose concentration determination; the glucose oxidase method was used. The consistency of the analyzer was tested using real blood samples twice before use. Serum insulin levels were measured using enzyme-linked immunosorbent assay (ELISA) with anti-insulin monoclonal antibody.

**Measurement of GLUT4 Protein Levels**

Three days after the OGTT, and 18 h after the last exercise bout with an immediate glucose intubation (1 g/kg BW), muscles were surgically removed for analysis of glycogen, and GLUT4 protein levels, and citrate synthase activity assay. Muscle samples for GLUT4 protein were homogenized in ice-cold HEPES (20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 1 mM EDTA, and 250 mM sucrose, pH 7.4) buffer (1:20) with a Polytron homogenizer (Kinematica, Littau, Switzerland). Sample homogenates and standards were diluted 1:1 with Laemmli sample buffer (125 mM Tris, 20% glycerol, 2% SDS, and 0.008% bromophenol blue, pH 6.8). The Western blotting procedure for GLUT4 analysis was followed the previously described method [17]. Muscle homogenates containing 75 µg (red gastrocnemius and plantaris muscles) of protein were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and electrophoretically transferred to a PVDF membrane. Two heart homogenates containing 15 and 30 µg of protein were loaded in parallel with the muscle samples. GLUT4 antiserum (Chemicon, Temecula, Calif., USA) was used for immunoblotting (directly against the carboxyl-terminus of the GLUT4 protein) in a dilution of 1:5,000. GLUT4 protein was visualized using an ECL Western blot detection