รายงานวิจัยฉบับสมบูรณ์

โครงการ

การศึกษาผลของการออกกำลังกายแบบต่อเนื่องต่อฤทธิ์ของฮอร์โมนอินซูลินในกล้ามเนื้อลายของหนูในภาวะเครียดแบบออกซิเดทีฟ

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1 กรกฎาคม 2547 – 30 มิถุนายน 2549

สัญญาเลขที่ MRG 4780115

รายงานวิจัยฉบับสมบูรณ์

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และสำนักงานกองทุนสนับสนุนการวิจัย

ABSTRACT

Project Code : MRG 4780115

Project Title : Effect of exercise training on insulin action in rat skeletal muscle subjected to oxidative stress

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Project Period : July 1st,2004 – June 30th, 2006

The direct effect of oxidative stress on insulin action on skeletal muscle glucose transport and the role of prior exercise training on insulin- stimulated glucose transport in skeletal muscle subjected to oxidative stress were investigated. Male Sprague-Dawley rats were randomly divided into either the sedentary control or the exercise-trained group. Rats in the latter group were trained by treadmill running for 6 weeks with moderate intensity. At the end of the exercise training program or of the sedentary period, type I soleus (SOL) and type II extensor digitorum longus (EDL) were isolated and incubated in the medium in the absence or presence of hydrogen peroxide (H_2O_2) and/or 5 mU/ml of insulin for two consecutive sessions of 60-min incubation. Thereafter, in vitro glucose transport activity, glutathione redox status (a principal representative of non-enzymatic antioxidant) and activities of antioxidant enzymes were examined.

H_2O_2 by itself activated ($p<0.05$) basal glucose transport activity (55% and 36%) and significantly inhibited ($p<0.05$) the action of insulin on glucose transport activity (28% and 22%) in the SOL and EDL, respectively, isolated from the sedentary animals. However, the inhibitory effects of H_2O_2 on insulin-stimulated skeletal muscle glucose transport were attenuated in the exercise-trained animals. Measurements of muscular glutathione redox status and activities of antioxidant enzymes showed no training effect.

Conclusively, oxidative stress directly inhibited insulin-stimulated glucose transport activity whereas prior exercise training can effectively protect against insulin resistance induced by oxidative stress. The underlying mechanism(s) responsible for this protective effect of exercise training on skeletal muscle insulin resistance, however, was not associated with adaptation of antioxidant systems and remain to be elucidated. Involvement of mitogen-activated protein kinases as an underlying mechanism should be further investigated.

Keywords: INSULIN RESISTANCE / EXERCISE / OXIDATIVE STRESS

บทคัดย่อ

รหัสโครงการ : MRG 4780115

ชื่อโครงการ : ผลของการออกกำลังกายแบบต่อเนื่องต่อฤทธิ์ของฮอร์โมนอินซูลิน ในกล้ามเนื้อลายของหนูในภาวะเครียดแบบออกซิเดทีฟ

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ระยะเวลาโครงการ 1 กรกฎาคม 2547 – 30 มิถุนายน 2549

การวิจัยนี้ได้ศึกษาผลโดยตรงของภาวะเครียดจากออกซิเดทีฟ (oxidative stress) ต่อการทำงานของฮอร์โมนอินซูลินในการกระตุ้นการขนส่งกลูโคสเข้ากล้ามเนื้อลาย และบทบาทของการออกกำลังกายอย่างต่อเนื่องต่อการทำงานของฮอร์โมนอินซูลินในการกระตุ้นการขนส่งกลูโคสเข้ากล้ามเนื้อลาย ภายใต้ภาวะเครียดจากออกซิเดทีฟ หนูขาวเพศผู้สายพันธุ์ Sprague-Dawley ได้ถูกแบ่งอย่างสุ่มออกเป็นสองกลุ่ม ได้แก่ กลุ่มควบคุมซึ่งไม่ได้ออกกำลังกาย และกลุ่มออกกำลังกายอย่างต่อเนื่อง หนูกลุ่มออกกำลังกายได้รับการฝึกฝนโดยวิ่งบนสายพานลู่วิ่งเป็นเวลา 6 สัปดาห์ สัปดาห์ละ 6 วัน วันละ 90 นาที ด้วยความหนักระดับปานกลาง เมื่อครบตามระยะเวลาที่กำหนด กล้ามเนื้อชนิดที่ 1 soleus และชนิดที่ 2 extensor digitorum longus (EDL) ได้ถูกผ่าตัดออกมา เพื่อนำมาทดสอบในสารละลายที่มีและไม่มีไฮโดรเจนเปอร์ออกไซด์ (H_2O_2) และ/หรือมีอินซูลิน (5 mU/ml) เป็นเวลา 2 ช่วง ช่วงละ 60 นาทีที่ต่อเนื่องกัน หลังจากนั้นได้ทำการวัดการขนส่งกลูโคสเข้ากล้ามเนื้อลาย, ตัวบ่งชี้สภาวะความเครียดจากออกซิเดทีฟภายในเซลล์ (glutathione redox status) และเอนไซม์ต้านอนุมูลอิสระ

ในหนูกลุ่มควบคุม H_2O_2 ออกฤทธิ์กระตุ้นการขนส่งกลูโคสเข้ากล้ามเนื้อลายในสภาวะที่ไม่มีอินซูลินอย่างมีนัยสำคัญทางสถิติ โดยกระตุ้นการขนส่งกลูโคสเข้ากล้ามเนื้อมากขึ้น 55% และ 36% สำหรับ soleus และ EDL ตามลำดับ แต่ในภาวะที่มีฮอร์โมนอินซูลิน H_2O_2 กลับออกฤทธิ์ยับยั้งการทำงานของฮอร์โมนอินซูลินอย่างมีนัยสำคัญทางสถิติเช่นกัน โดยลดการทำงานของฮอร์โมนอินซูลินในการกระตุ้นการขนส่งกลูโคสเข้ากล้ามเนื้อ soleus 28% และ EDL 22% อย่างไรก็ตาม ฤทธิ์ในการยับยั้งการทำงานของฮอร์โมนอินซูลินโดย H_2O_2 นี้ ไม่เกิดขึ้นในกล้ามเนื้อของหนูกลุ่มออกกำลังกายอย่างต่อเนื่อง แต่ค่าตัวบ่งชี้สภาวะความเครียดจากออกซิเดทีฟภายในเซลล์ และเอนไซม์ต้านอนุมูลอิสระในกล้ามเนื้อของหนูกลุ่มออกกำลังกายอย่างต่อเนื่องไม่เปลี่ยนแปลงเมื่อเปรียบเทียบกับหนูกลุ่มควบคุม

กล่าวโดยสรุป ความเครียดจากออกซิเดทีฟออกฤทธิ์โดยตรงมีผลในการยับยั้งการทำงานของฮอร์โมนอินซูลินในการกระตุ้นการขนส่งกลูโคสเข้ากล้ามเนื้อลาย ในขณะที่การออกกำลังกายอย่างต่อเนื่อง สามารถป้องกันภาวะดื้อต่อฮอร์โมนอินซูลินของกล้ามเนื้อลายที่เกิดจากภาวะเครียดจากออกซิเดทีฟได้อย่างมีประสิทธิภาพ กลไกระดับเซลล์ซึ่งส่งผลให้การออกกำลังกายอย่างต่อเนื่องสามารถป้องกันภาวะดื้อต่อฮอร์โมนอินซูลินของกล้ามเนื้อลายนั้น ไม่เกี่ยวข้องกับระบบต้านภาวะเครียดจากออกซิเดทีฟภายในเซลล์และยังเป็นประเด็นสำคัญที่จะต้องทำการศึกษาต่อไป

คำหลัก: INSULIN RESISTANCE / EXERCISE / OXIDATIVE STRESS

INTRODUCTION

Insulin is the predominant hormone responsible for the maintenance of glucose homeostasis through its regulation of metabolic activities in muscle, liver, and adipose tissue. Skeletal muscle, which makes up about 40% of the body mass of humans and other mammalian species, is the major tissue responsible for the peripheral disposal of glucose in the face of a glucose or insulin challenge or during an exercise bout (1,2). Insulin stimulates glucose uptake by rapidly mobilizing preexisting glucose transporters (GLUT-4) from an intracellular storage organelle to the plasma membrane.

Insulin resistance of skeletal muscle glucose transport represents a major defect in the normal maintenance of euglycemia (3,4) and is often accompanied by a variety of metabolic abnormalities, including obesity, dyslipidemia, type 2 diabetes, hypertension, and atherosclerosis (5,6). Resistance to insulin of skeletal muscle glucose transport could result from any of the following possibilities: defects in insulin signaling and/or metabolic defects such as increased levels of plasma free fatty acid. More recently, evidence suggest that oxidative stress, defined as a persistent imbalance between the production of highly reactive oxygen species and antioxidant defenses, is a novel candidate. Because of the ability to directly oxidize and damage DNA, protein, and lipid, reactive oxygen species are believed to play a key direct role in a number of pathogenesis including the progression of skeletal muscle insulin resistance.

Furthermore, free radical-mediated oxidative damage has been observed in a variety of pathological conditions, including insulin resistance and type 2 diabetes. Baynes (1991) has postulated that increased formation of and reduced removal of reactive oxygen species and free radicals leads to accelerated lipid peroxidation and membrane destruction (7). Several investigations have shown that in diabetes both increased reactive oxygen species production and a reduced antioxidant defense capacity develops, resulting in an impaired oxidant-antioxidant status (7-11). In addition, increased serum levels of several oxidation end-products have been described in type 1 and type 2 diabetic patients, as well as in several experimental animal models of diabetes (12,13). These results suggest that increased oxidative stress is a common surrogate of the insulin-resistant, diabetic state.

To date, the importance of oxidative stress-related processes, contributing to the metabolic abnormalities of diabetes and particularly to the complex and yet incompletely-defined pathogenesis of insulin resistance, remains unclear. Although numerous clinical and

epidemiological studies support a connection between oxidative stress and insulin resistance, direct experimental evidence for a cause and effect relationship between oxidative stress and insulin resistance is limited. Using 3T3-L1 adipocytes, Rudich et al. reported that prolonged exposure to low-grade oxidative stress markedly decreased insulin-stimulated glucose metabolism (14). This decreased insulin responsiveness was associated with decreased GLUT-4 protein and GLUT-4 mRNA. Whereas these results are consistent with the hypothesis that oxidative stress can directly and negatively impact insulin action on glucose transport, these findings arrive from studies using cell lines and it remains unclear how oxidative stress modulates insulin action in mammalian tissues.

Saengsirisuwan et al. demonstrated in the insulin-sensitive muscle of the lean Zucker rat that while an oxidative stress induced by hydrogen peroxide can activate basal glucose transport in both type I soleus and type II epitrochlearis muscles (30% and 48%, respectively), this same oxidative stress significantly inhibits the insulin-mediated enhancements in glucose transport by 75% and 55%, respectively (15). This observation supports a direct role of oxidative stress in the induction of insulin resistance in mammalian skeletal muscle.

Additionally, Saengsirisuwan et al. have demonstrated that the levels of protein carbonyls (an index of oxidative stress) in the soleus, plantaris, and cardiac muscles from the insulin-resistant rat are markedly higher than those in the same tissues from the insulin-sensitive rat, supporting the potential role of oxidative stress in the etiology of insulin resistance (16). Moreover, they show that a reduction in muscle protein carbonyl levels is achieved in the obese rats that underwent exercise training, and the reduction of protein carbonyl level in the muscle of the exercise-trained obese animals is significantly related with the improvement in insulin-mediated glucose transport. These findings clearly address the potential utility of exercise training to diminish oxidative stress and improve insulin sensitivity in insulin-resistant skeletal muscle of the obese rat. It is, however, currently unknown whether exercise training would enable to protect against skeletal muscle insulin resistance induced by oxidative stress. *Therefore, the main objective of the present investigation was to assess the potential protective effect of exercise training against an induction of insulin resistance in rat skeletal muscle by oxidative stress.*

Antioxidant systems are responsible for cellular protection against oxidative stress. They can be divided into two main categories including non-enzymatic and enzymatic antioxidants. Reduced glutathione (GSH) is a major intracellular non-enzymatic antioxidant in cell and is a substrate for glutathione peroxidase enzyme, which catalyzes the reduction of hydrogen or lipid

peroxides. By donating a pair of electrons to a hydroperoxide, two GSH molecules are oxidized to glutathione disulfide. Thus, analysis of the ratio of GSH / oxidized glutathione content of cells can provide a valid index of cellular oxidant status.

It is well documented that an acute bout of exhaustive exercise induces the production of reactive oxygen species and markers of oxidative stress in muscle of animal models (17) and in humans (18). In contrast to acute bout of exercise, chronic exercise training has been shown to increase antioxidant defense systems in skeletal muscle, including GSH content (19,20). In regards to the experimental model in the present investigation, the effect of direct exposure of oxidative stress to skeletal muscle on cellular oxidant status was evaluated. This study also assessed the effectiveness of exercise training on the enhancement of cellular oxidant status and whether the adaptation of cellular oxidant status, if any, was adequate to protect the cell from an invasion of oxidative stress.

Two major muscular antioxidant enzymes, glutathione peroxidase (GPX) and catalase (CAT), were investigated in the present study because their functions directly involve in the elimination of H_2O_2, an influential oxidative stress used in the current experiment. Even though the effects of exercise training on the enzymatic antioxidant system are still controversial, there are studies (19,20) supporting the notion that exercise training can enhance antioxidant enzyme activity as prolonged muscular exertion continuously stimulates production of reactive oxygen species and other oxidative stress which consequently leads to adaptation of the antioxidant defense system.

Taken together, the objectives of the present study were to:

1. Determine the effect of oxidative stress on insulin-stimulated glucose uptake in skeletal muscle isolated from the Sprague-Dawley rats.
2. Examine the effect of oxidative stress on the oxidant status in skeletal muscle.
3. Evaluate how endurance exercise training modulates insulin action on glucose transport rate in skeletal muscle subjected to oxidative stress.
4. Assess the effect of endurance exercise training on muscular antioxidants systems and on the oxidant status in skeletal muscle subjected to oxidative stress.

METHODS

1. Animals

Male Sprague-Dawley rats (n = 122) were purchased from the Laboratory Animal Center (Salaya Campus, Mahidol University, Thailand). The animals were obtained at the age of 4-wk old (weighing 90-120 g). Rats were housed separately in hygienic stainless steel hanging cages at the animal care unit of the Faculty of Science, Phayathai campus, Mahidol University. Condition in the housing-room was tightly controlled at 22°C with relative humidity at 60%-70% and with a normal 12-hr dark-light cycle. Animals were allowed to get used to the new ambient for 5 days and had free access to water and rat chow. Rats were randomly assigned to either an exercise-trained group or an untrained sedentary control group. Animals in the exercise-trained group ran on a motorized treadmill based on the endurance training protocol modified from Slentz *et al*. (21).

During the first week, rats were familiarized to treadmill running at 15-20 m/min at 0% grade for 10-20 min/day; 6 days/wk. Speed and duration of running were gradually increased to 25m/min at 10% grade for 40-70 min/day by the end of week 3. During week 4-6, running speed and duration were kept constant at 20-25 m/min at 5-15% grade for 90 min/day. The exercise intensity used in this study was in a range of moderate intensity (60% - 70% VO_{2max}) according to Bedford *et al*. (22). During exercise training period, room-temperature air blow were used so as to stimulate the continuous running of the rodents.

Body weight was measured weekly in order to confirm that the animal in both groups have normal growth rate. On the experimental day after a 12-hr overnight fasted, weight of each rat was measured and recorded. The incubation experiment was initiated at 8 AM after an overnight food restriction. Animals were weighed and deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). Both sides of soleus and EDL muscles were dissected and prepared for *in vitro* incubation.

2. Exposure of Muscle to Oxidative Stress

The soleus and extensor digitorum longus (EDL) were isolated from the anesthetized animals. Each muscle was carefully divided into two strips. One soleus and one EDL strip were incubated in the absence or presence of 100 mU/ml glucose oxidase (in order to generate H_2O_2 and cause oxidative stress), and the contralateral soleus muscle and second EDL strip were

incubated in the absence or presence of oxidative stress with an addition of a maximally effective concentration of insulin (5mU/ml). Muscles were incubated for two consecutive periods of 60-min at 37°C in 3 ml oxygenated (95% O_2 - 5%CO_2) Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA. After the initial incubation period, the muscles were rinsed for 10 min at 37°C in 3 ml oxygenated KHB containing 40 mM mannitol, 0.1% BSA.

3. Measurements of Experimental Parameters

Indices of systemic change and metabolic adaptation in skeletal muscle as a result of endurance-exercised training.

Heart weight to body weight ratio was used as an index of systemic adaptation. Heart was dissected out from the animal after muscle isolation was finished. Blood remained inside the heart was drained out by opening the heart chamber by scissors and the heart was dried by putting on filter papers. The heart was frozen by liquid nitrogen and weighed immediately. Metabolic adaptation in skeletal muscle was represented by the activity of citrate synthase enzyme. Citrate synthase activity was determined according to Srere (23).

***In vitro* glucose transport activity**

After prior incubation, muscle strips were transferred to 2 ml KHB, containing 1 mM 2-[1,2-^{3}H] deoxyglucose (2-DG, 300 mCi/mmol), 39 mM [U-^{14}C] mannitol (0.8 mCi/mmol), 0.1%BSA, and insulin, if previously present. At the end of this final 20-min incubation period at 37°C, the muscles were removed, trimmed of excess fat and connective tissue, quickly frozen, and weighed. The frozen muscles were dissolved in 0.5 ml of 0.5 N NaOH. After the muscles were completely solubilized, 10 ml of scintillation cocktail were added and the specific intracellular accumulation of 2-DG were determined as described previously (24).

Determination of H_2O_2 concentration

The concentration of H_2O_2 in the medium incubated with muscle strip was measured immediately at the end of the first and second incubation period so as to assess the H_2O_2 scavenging capacity of the muscle isolated from the sedentary and the exercise-trained animals. After the muscle was taken out for tissue termination, 1 ml of the incubation medium was drawn and mixed with 0.25 ml of 50% (w/v) trichloroacitic acid and chilled on ice for 20 minutes. Thereafter, the mixture was centrifuged at 5,000 g (25°C) for 10 min. The supernatant was utilized to determine H_2O_2 concentration by spectrophotometric method according to Thurman *et al*. (25).

Indicator of oxidant status

In order to assess the oxidant status in the muscles, a marker of a disturbed oxidant-antioxidant balance is required. This measurement determines the impact of oxidative stress as well as exercise training on muscle oxidant status and relates the oxidant status to insulin action in skeletal muscle. Muscle samples were analyzed to determine the ratio of reduced glutathione to oxidized glutathione (RGSH/GSSG). Total glutathione were determined according to the glutathione reductase-DTNB recycling procedure described by Tietze (26), and oxidized glutathione were analyzed by the method of Griffith (27).

Antioxidant enzymes

Measurements of the activities of GPX and CAT, the major H_2O_2 – eliminating enzymes were done as follows. The portion of muscle (∼ 90 mg) was homogenized in an ice-cold medium containing 0.25 M sucrose, 10 mM 2-amino-2-hydroxymethyl-1,3-propanediol (Tris), and 0.1 mM EDTA (pH 7.4, wt/vol 1: 7). The homogenate was centrifuged at 750 g (4°C) for 15 min., and the supernatant was used for GPX and CAT assays. GPX activity was assayed spectrophotometrically according to Tappel (28). CAT activity was determined by the method of Aebi (29).

4. Statistical Analysis

Data were presented as means $\pm$ SEM. Statistical analysis was undertaken using paired or unpaired Student's *t*-test. Differences between groups were considered statistically significant when $p < 0.05$.

RESULTS

1. Characteristics of Sedentary Control and Exercise-Trained Rats

General characteristics of sedentary and exercise-trained animals are shown in Table 1. Initial and final body weights between both groups were not significantly different. Heart weight of the exercise-trained group was significantly heavier than that of the sedentary control group. Ratio of heart weight to body weight of the animals that received exercise training was significantly higher than that of the sedentary control group as well. The result indicated hypertrophy of the heart in the exercise-trained animals. Moreover, exercise training significantly enhanced activities of an oxidative enzyme, citrate synthase, in the soleus muscle. Taken together with cardiac hypertrophy, a significant increase in activity of citrate synthase enzyme confirmed that the exercise training protocol effectively brought about physiological adaptations.

Table 1. General characteristics of the sedentary control and exercise-trained rats

	Animals	
Parameters	Sedentary control	Exercise-trained
Initial body wt (g)	172.4±4.3	159.3± 4.3
Final body wt (g)	360.4±5.5	359.3± 4.5
Heart wt (g)	1.07±0.02	1.16±0.01**
HW/BW (g/kg)	2.97±0.07	3.23±0.05**
Citrate synthase activities (nmol/μg P/min)	0.14±0.01	0.25±0.03*

Values are means ± SEM.* $p<0.05$, ** $p<0.01$ sedentary vs. exercise-trained groups.

2. Measurement of Muscle Glucose Transport Activities

H_2O_2 is one of biologically important oxidant because of its high ability to diffuse through hydrophobic membrane like cell membrane and its role on initiation of free radical cytotoxicity. In this experimental setup, H_2O_2 was generated by adding glucose oxidase (100 mU/ml) into the incubation medium. The effects of oxidative stress on basal and insulin-stimulated glucose transport rates in type I slow-oxidative soleus muscle are presented in Figure 1 (upper panel). *In vitro* rates of 2-deoxyglucose (2-DG) uptake were presented in the unit of pmol/mg wet muscle weight/ 20 min. Basal glucose transport rate in the soleus muscle isolated from the sedentary control animals was 192.2$\pm$9.5 pmol/mg/20min. We found that in the absence of oxidative stress, insulin significantly stimulated muscle glucose transport (465.8$\pm$19.9 pmol/mg/20min). H_2O_2 by itself activated ($P<0.05$) basal glucose transport activity by 55% (192.2$\pm$9.5 vs. 297.8$\pm$20.0 pmol/mg/20min). On the other hand, it significantly inhibited the action of insulin on glucose transport activity by approximately 28% (465.8$\pm$19.9 vs. 340.9$\pm$19.8 pmol/mg/20min).

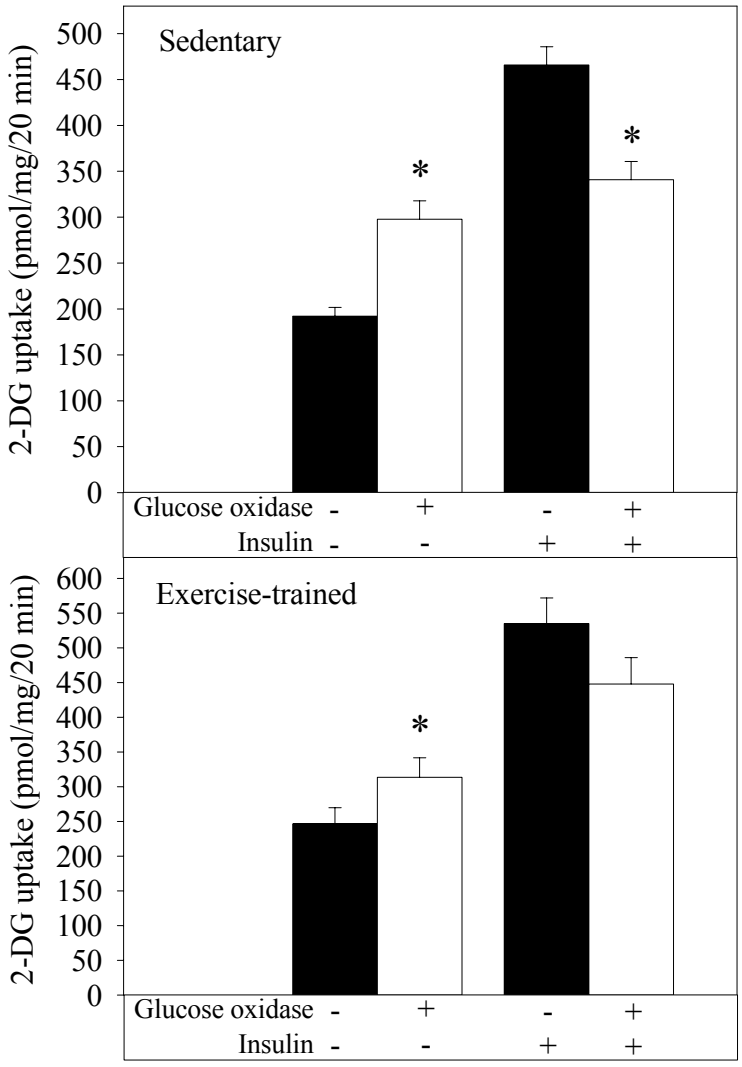

Figure 1. *In vitro* rates of 2-deoxyglucose uptake in the soleus muscle from the sedentary control (upper panel) and the exercise-trained (lower panel) animals in the absence and presence of glucose oxidase (100 mU/ml) and /or insulin (5 mU/ml). Values are means ± SEM. * $p<0.05$ absence vs. presence of glucose oxidase.

Results of the role of oxidative stress on basal and insulin-stimulated 2-DG transport activities in type II extensor digitorum longus (EDL) of the sedentary control animals were similar to those in the soleus muscle even though the degree of glucose transport into EDL muscle was lower than that of the soleus muscle. Basal glucose transport into EDL muscle in sedentary control group was 165.5±10.4 pmol/mg/20min and insulin-stimulated muscle glucose transport was 331.5±22.8 pmol/mg/20min. Demonstrated in Figure 2 (upper panel), while H_2O_2 significantly activated basal glucose transport by 36% (165.5±10.4 vs. 225.5±19.3 pmol/mg/20min), it significantly decreased the action of insulin on glucose transport activity by 22% (331.5±22.8 vs. 261.7±18.0 pmol/mg/20min).

In order to answer whether endurance exercise training can protect against this inhibitory effect of oxidative stress on insulin action, the effect of oxidative stress on glucose transport activity was determined in the muscle isolated from the rats that underwent endurance exercise training. Figure 1 (lower panel) illustrates the glucose transport activity into the soleus muscle of the exercise-trained animals. Basal glucose transport into the soleus in this experimental group was 246.6±23.1 pmol/mg/20min. In the exercise-trained soleus, insulin significantly stimulated a great deal of glucose transport activity (534.8±37.2 pmol/mg/20min). Similar to the sedentary control group, oxidative stress significantly stimulated basal glucose transport activity (313.7±27.8 pmol/mg/20min). Interestingly, the inhibitory effect of oxidative stress on insulin-stimulated muscle glucose transport was prevented in the muscle from the rat that underwent exercise training (448.0±37.9 pmol/mg/20min). The protective effect of exercise training on insulin action on muscle glucose transport was also observed in type II EDL muscle (Figure 2, lower panel). Basal glucose transport activity of the EDL muscle from exercise-trained group was 196.8±17.9 pmol/mg/20min. Not surprisingly, insulin alone significantly stimulated glucose uptake to 372.6±29.1 pmol/mg/20min. Despite a lesser degree, H_2O_2 alone also increased glucose transport rate to 274.3±17.6 pmol/mg/20min. In contrast to the inhibitory effect of H_2O_2 on insulin action in the EDL muscle of the sedentary animals, the presence of H_2O_2 did not inhibit glucose transport activity of the exercise-trained EDL muscle in the insulin-stimulated condition (372.6±29.1 pmol/mg/20min vs. 342.7±29.5 pmol/mg/20min).

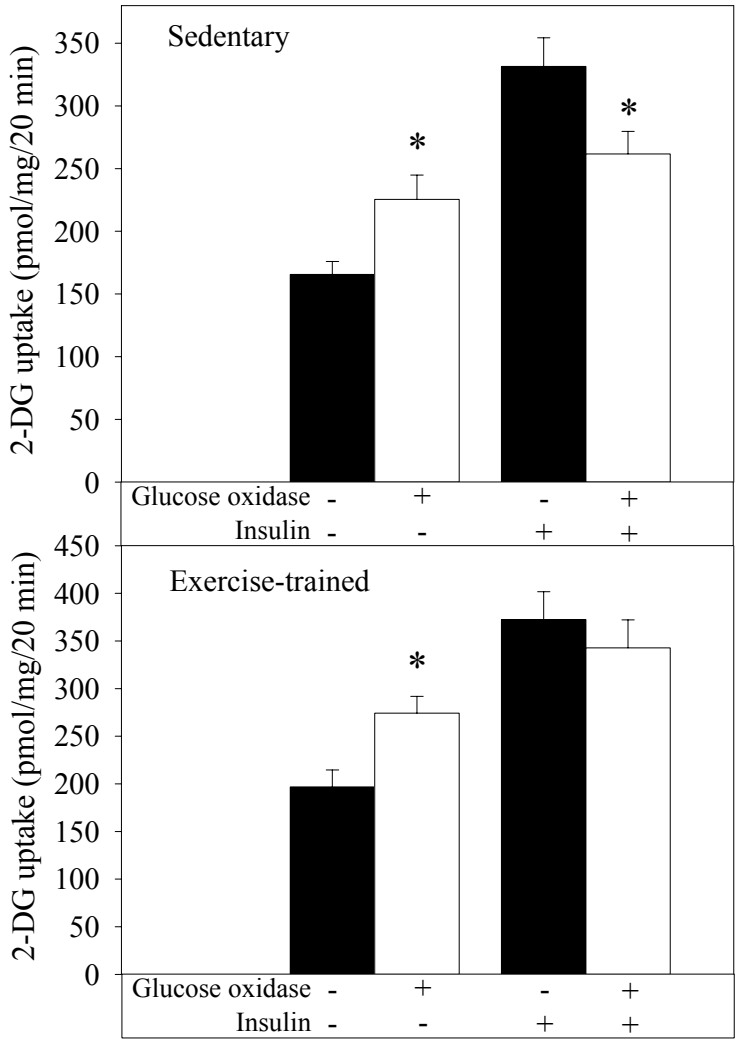

Figure 2. *In vitro* rates of 2-deoxyglucose uptake in the extensor digitorum longus muscle from the sedentary control (upper panel) and the exercise-trained (lower panel) animals in the absence and presence of glucose oxidase (100 mU/ml) and /or insulin (5 mU/ml). Values are means ± SEM. * $p<0.05$ absence vs. presence of glucose oxidase.

3. Concentration of H_2O_2 in the Medium Following Tissue Incubation

The concentration of H_2O_2 in the incubation medium was determined right after the muscle was taken out for tissue termination in order to evaluate the H_2O_2 scavenging capacity of the muscle from the sedentary and the exercise-trained animals. Concentration of H_2O_2 at the end of the first 60-min incubation period is shown in Figure 3. In the sedentary control animals (filled bars), the H_2O_2 concentrations in the medium incubated with soleus muscle were lower than those incubated with EDL muscle. The results revealed the fact that the high-oxidative soleus muscle posses a higher antioxidant capacity than the predominantly glycolytic EDL muscle. Compared to the sedentary group, the concentrations of H_2O_2 in the medium incubated with exercise-trained muscles tended to be lower in most conditions. A significant difference was observed in the medium incubated with the soleus for 60 min. Following the first 60-min exposure to H_2O_2 in the soleus muscle, the levels of H_2O_2 concentration in the absence of insulin were 0.097$\pm$0.018 μM/mg muscle tissue in the sedentary control group and 0.063$\pm$0.013 μM/mg muscle tissue in the exercise-trained group while H_2O_2 concentrations in the presence of insulin were 0.087$\pm$0.015 μM/mg muscle tissue in the sedentary control group and 0.037$\pm$0.011 μM/mg muscle tissue in the exercise-trained group. In the medium containing H_2O_2 and incubated with EDL muscle, the H_2O_2 concentrations were 0.170$\pm$0.043 μM/mg muscle tissue and 0.074$\pm$0.007 μM/mg muscle tissue in the sedentary and the exercise-trained groups, respectively. In the presence of insulin, H_2O_2 concentrations were 0.230$\pm$0.049 μM/mg muscle tissue in the sedentary group and 0.172$\pm$0.019 μM/mg muscle tissue in the exercise-trained group.

At the end of the second 60-min incubation period (Figure 4), H_2O_2 concentrations remained in the medium of all conditions were higher than those after first 60-min incubation period. However, the pattern and tendency of the H_2O_2 concentrations at the end of the first and the second 60-min incubation period were similar. The H_2O_2 concentrations in the medium incubated with soleus muscle in the absence of insulin were 1.52$\pm$0.56 μM/mg muscle tissue and 0.82$\pm$0.13 μM/mg muscle tissue in the sedentary and the exercise-trained groups, respectively. While H_2O_2 concentrations in the presence of insulin were 1.60$\pm$0.42 μM/mg muscle tissue in the sedentary control group and 1.27$\pm$0.31 μM/mg muscle tissue in the exercise-trained group. Levels of H_2O_2 concentrations in the medium containing H_2O_2 and incubated with EDL muscle were 3.23$\pm$1.11 μM/mg muscle tissue in the sedentary group and 2.36$\pm$0.62 μM/mg muscle tissue in the exercise-trained group. In the presence of insulin, H_2O_2

concentrations were 3.84±1.08 μM/mg muscle tissue in the sedentary group and 3.38±0.76 μM/mg muscle tissue in the exercise-trained group.

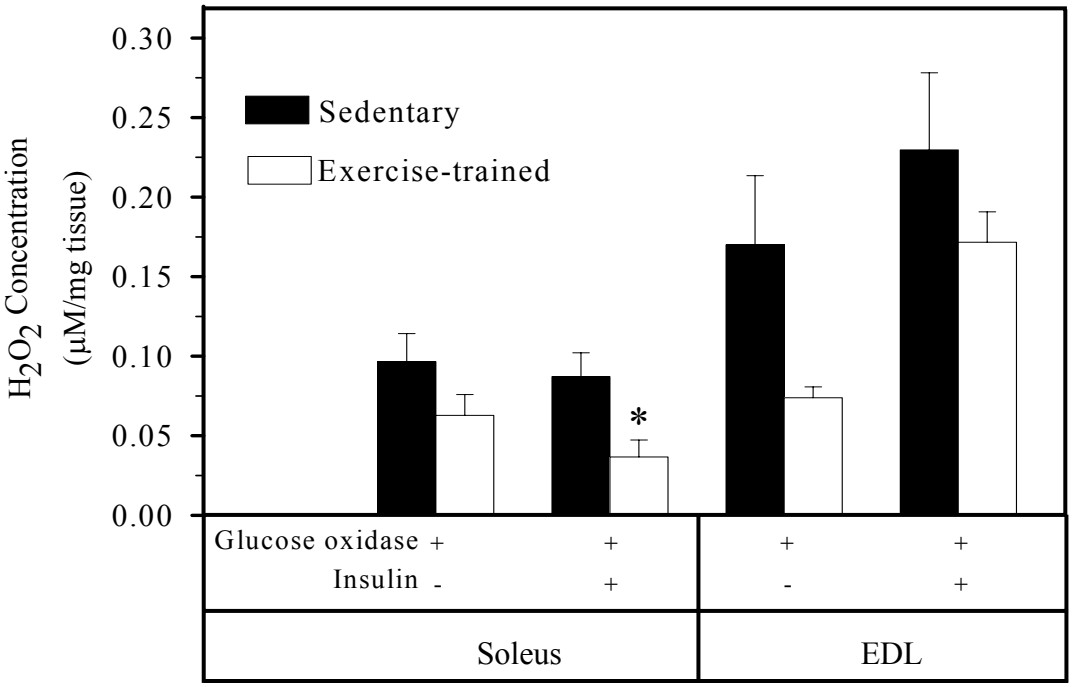

Figure 3. Concentration of hydrogen peroxide (expressed in a unit of micromolar per milligram muscle tissue) in the incubation medium in the presence of 100 mU/ml glucose oxidase and/or 5 mU/ml insulin following the first 60-min incubation period with the soleus or the extensor digitorum longus muscles from the sedentary control (filled bars) or the exercise-trained animals (unfilled bars). Values are means ± SEM for 7 sedentary and 7 exercise-trained animals. * $p<0.05$, sedentary vs. exercise-trained groups.

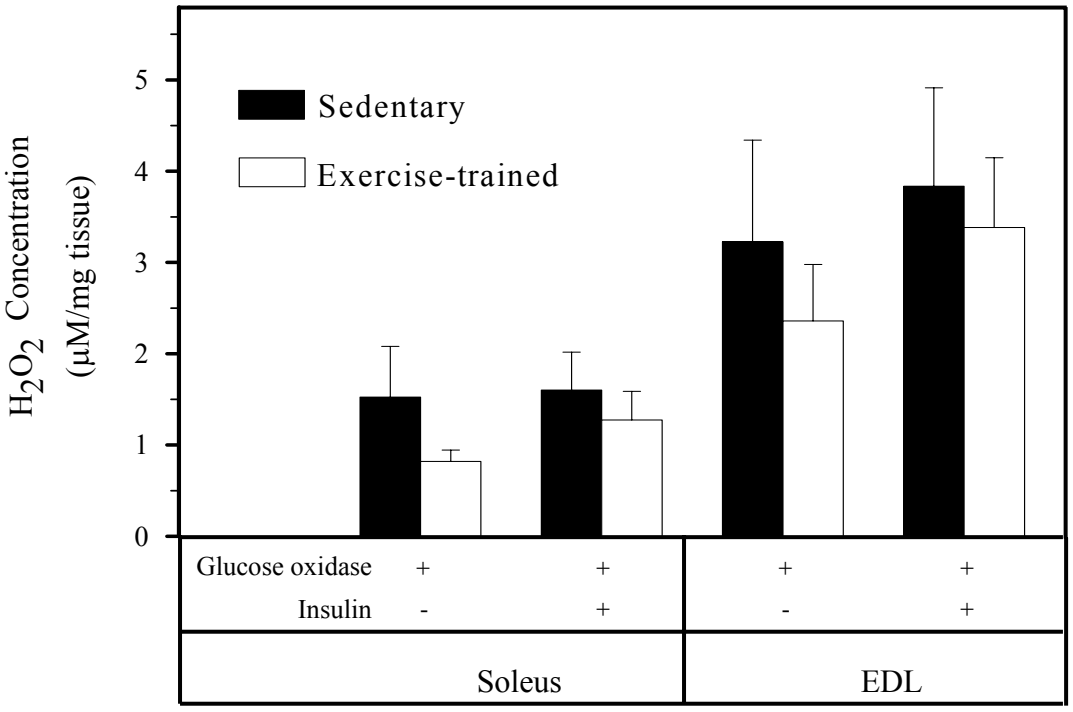

Figure 4. Concentration of hydrogen peroxide (expressed in a unit of micromolar per milligram muscle tissue) in the incubation medium in the presence of 100 mU/ml glucose oxidase and/or 5 mU/ml insulin following the second 60-min incubation period with the soleus or the extensor digitorum longus muscles from the sedentary control (filled bars) or the exercise-trained animals (unfilled bars). Values are means ± SEM for 7 sedentary and 7 exercise-trained animals.

4. Effect of Exercise Training on Antioxidant Systems

To test whether antioxidant capacity involves in the underlying mechanism responsible for the protective effect of exercise training on impaired insulin action caused by oxidative stress, parameters in the antioxidant systems including the activities of the antioxidant enzymes and the contents of glutathione as well as glutathione redox status were investigated.

4.1 Activities of the antioxidant enzymes

GPX activities in the soleus and EDL muscles of rats are shown in Figure 5. In sedentary control animals, GPX activity of the soleus was approximately four folds higher than that of the EDL muscle. Values of GPX activity in the soleus and EDL muscles from the sedentary control group were 111.0±8.7 and 25.4±6.0 units/mg protein, respectively. Exercise training, however, did not enhance the GPX activity in both the soleus and EDL muscles. GPX activities in muscles from the exercise-trained soleus and EDL muscles were 95.2±4.8 and 38.7±1.9 units/mg protein, respectively.

Figure 5. Glutathione peroxidase (GPX) activity in rat soleus and extensor digitorum longus muscles. Means ±SEM are presented; n = 6 in the sedentary control group and n = 4 in the exercise-trained group.

Figure 6 depicts an activity of catalase. Similar to GPX activity, the soleus muscle in the sedentary control group contained a higher catalase activity than the EDL muscle approximately by four times. Catalase activity was 2.24±0.18 K x 10^{-2} in the soleus and 0.59±0.11 K x 10^{-2} in EDL muscle. Nevertheless, there was no significant difference in catalase activity between groups in both types of muscle. Levels of catalase activity in soleus and EDL muscles from the exercise-trained animals were 2.31±0.29 K x 10^{-2} and 0.88±0.11 K x 10^{-2}, respectively.

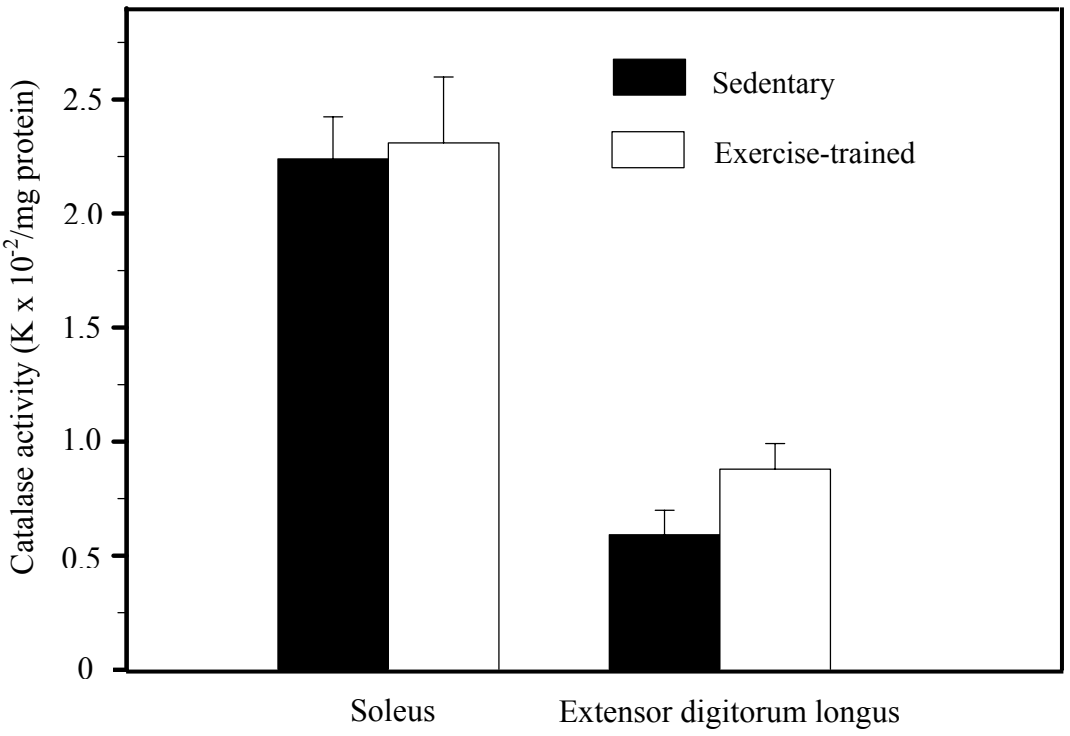

Figure 6. Catalase activity in rat soleus and extensor digitorum longus muscles. Means ± SEM are presented; n = 5 in sedentary control group and n = 4 in exercise-trained group.

4.2 Glutathione Contents and Glutathione Redox Status

Glutathione contents and glutathione redox status (as reflected by the ratio of reduced glutathione to oxidized glutathione (RGSH/GSSG) in soleus muscle are shown in Figure 7. A higher RGSH/GSSG ratio reflects a better glutathione redox status. Contents of TGSH, RGSH, and GSSG were not significantly different between the sedentary control and the exercise-trained groups. Likewise, glutathione redox status was not influenced by exercise training.

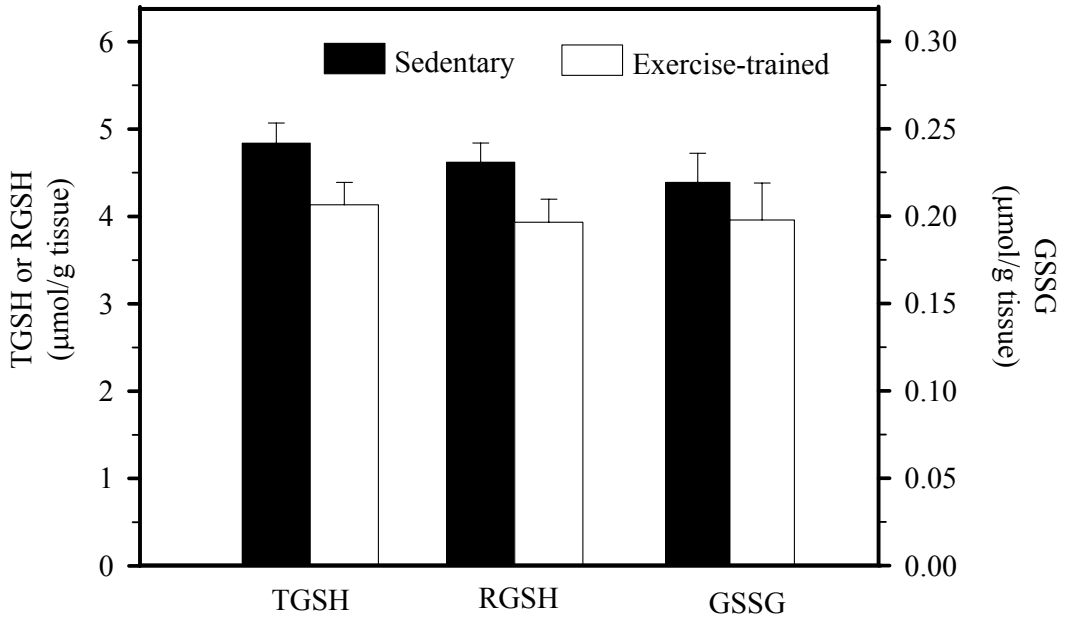

Figure 7. Total glutathione (TGSH), reduced glutathione (RGSH), glutathione disulfide (GSSG) in soleus of sedentary (filled bars) and exercise-trained rats (unfilled bars). Means ± SEM are presented; n = 10 in each group.

In the EDL muscle, the level of TGSH, RGSH, and GSSG contents in sedentary control were less than those in soleus 3.5, 3.5, and 3.0 folds respectively. Like the soleus muscle, exercise training did not significantly modulate any values of the glutathione contents and the glutathione redox status. Figure 8 illustrates the data by histogram.

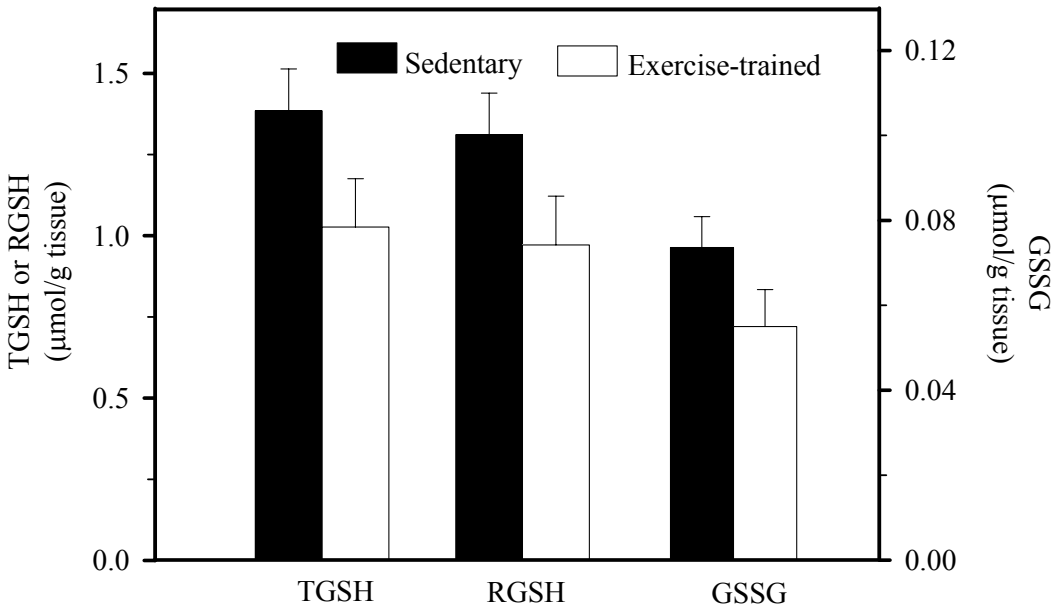

Figure 8. Total glutathione (TGSH), reduced glutathione (RGSH), glutathione disulfide (GSSG) in extensor digitorum longus of sedentary (filled bars) and exercise-trained rats (unfilled bars). Means ± SEM are presented; n = 11 in each group.

5. Effects of Oxidative Stress and Exercise Training on Glutathione Contents and Glutathione Redox Status

The glutathione redox status was also determined in the muscle following incubation with 100 mU/ml glucose oxidase and /or 5 mU/ml insulin. Figures 9, 10, and 11 demonstrate the contents of TGSH, RGSH, and GSSG, respectively. The values obtained from the incubated soleus muscle from the sedentary group are shown in the figures on the left panel and those data from the exercise-trained group are illustrated in the figures on the right panel. The TGSH contents in soleus muscle incubated in the absence of glucose oxidase and insulin, in the presence of glucose oxidase alone, in the presence of insulin alone, and in the presence of both glucose oxidase and insulin were 3.05±0.34, 2.64±0.17, 2.71±0.15, and 2.50±0.26 μmol/g muscle wet weight, respectively. RGSH contents in soleus muscle incubated in the same four conditions of the incubation medium were 2.92±0.32, 2.52±0.17, 2.61±0.14, and 2.39±0.24 μmol/g muscle wet weight, respectively. According to the same order of the four incubation conditions, the GSSG contents in the incubated soleus muscle were 0.131±0.024, 0.119±0.012, 0.101±0.010, and 0.109±0.019 μmol/g muscle wet weight, respectively.

The contents of TGSH, RGSH, and GSSG in the exercise-trained soleus muscle were not significantly different from those obtained from the untrained muscle. For the exercise-trained animals, the TGSH contents in soleus muscle incubated in the four conditions were 3.02±0.36, 2.61±0.43, 2.69±0.32, and 2.49±0.30 μmol/g muscle wet weight, respectively. The RGSH contents in soleus muscle incubated in the experimental conditions were 2.92±0.35, 2.51±0.42, 2.59±0.31, and 2.39±0.30 μmol/g muscle wet weight, respectively. Values of the GSSG contents in the incubated soleus muscle were 0.107±0.017, 0.093±0.017, 0.092±0.013, and 0.102±0.015 μmol/g muscle wet weight, respectively.

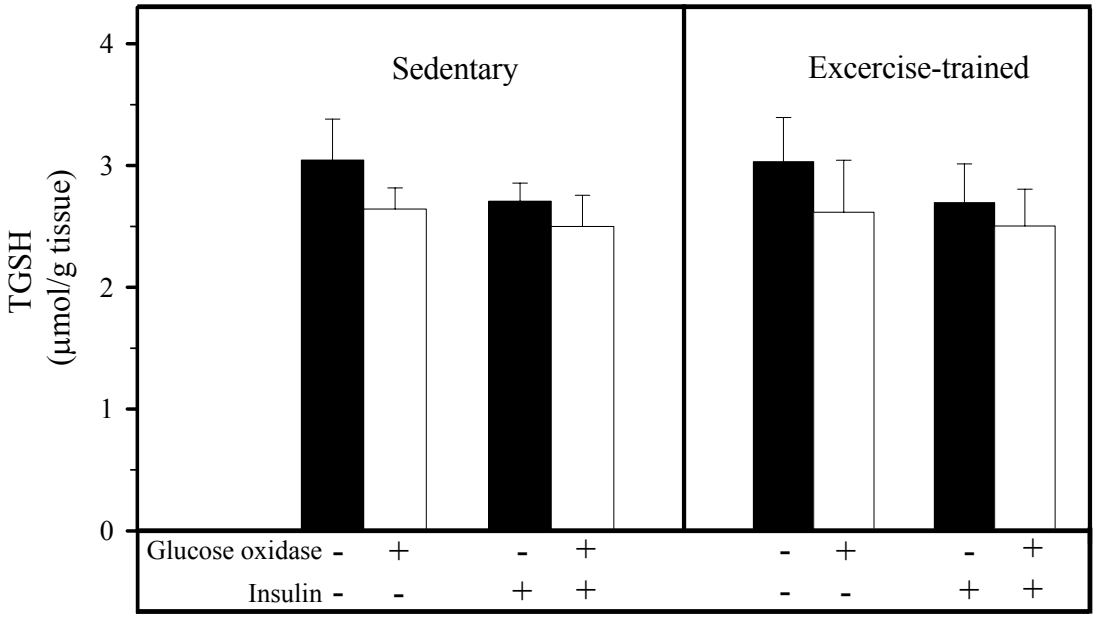

Figure 9. Total glutathione contents (µmol/g tissue) in incubated soleus muscle of rats in sedentary control and exercise-trained groups in the absence and presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means ± SEM; n = 9 in each group.

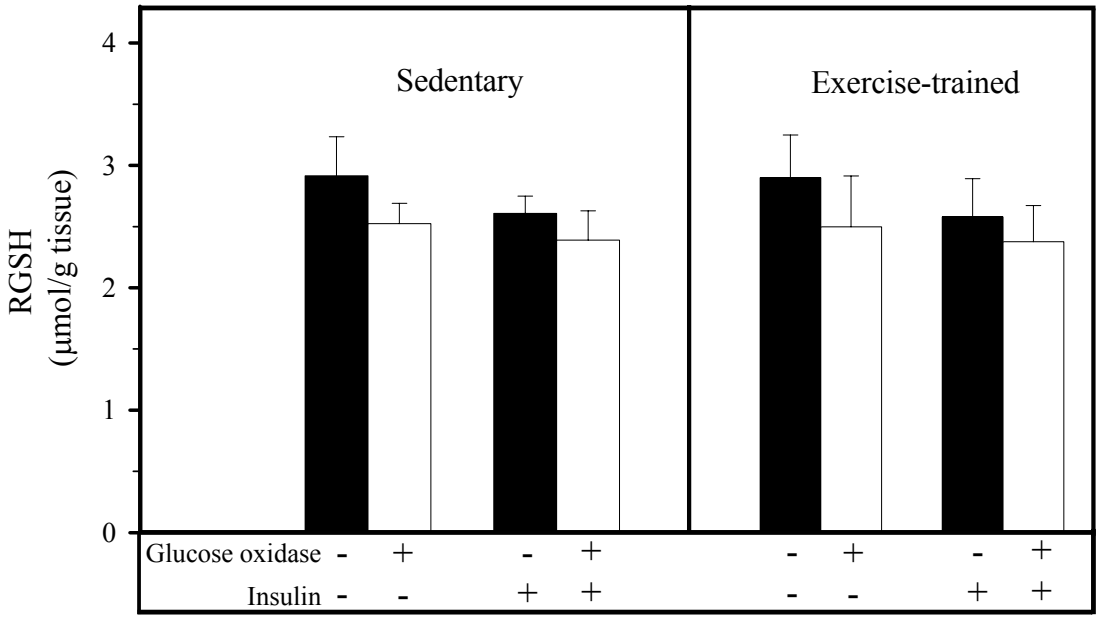

Figure 10. Reduced glutathione contents (µmol/g tissue) in incubated soleus muscle of rats in sedentary control and exercise-trained groups in the absence and presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means ± SEM; n = 9 in each group.

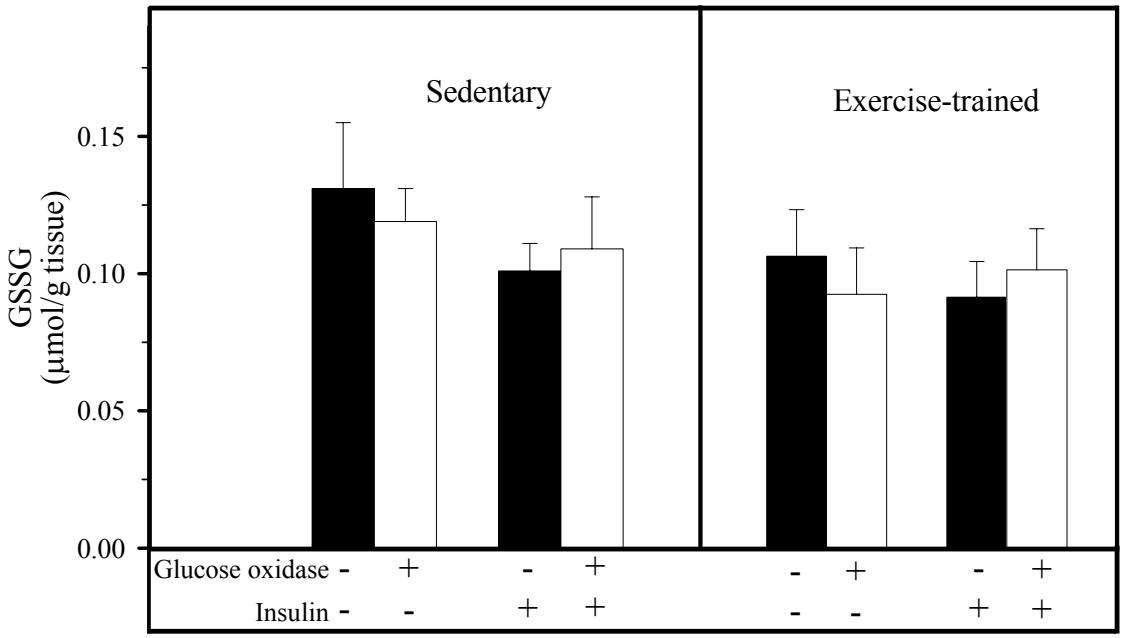

Figure 11. Oxidized glutathione contents (µmol/g tissue) in incubated soleus muscle of rats in sedentary control and exercise-trained groups in the absence and presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means ± SEM; n = 9 in each group.

Despite a lack of statistical significance, the RGSH/GSSG ratio of the muscle obtained from the sedentary animals tended to be reduced by oxidative stress (Figure 12, left panel). The ratios of RGSH/GSSG in soleus muscle incubated in the four incubation conditions were 26.9±3.9, 22.2±1.7, 26.9±1.8, and 25.2±3.0, respectively. Interestingly, exercise training had no effect on the glutathione redox status caused by oxidative stress (Figure 12, right panel). The ratios of RGSH to GSSG in soleus muscle incubated in the four experimental conditions were 29.0±2.0, 31.5±5.7, 32.6±5.0, and 27.0±4.9, respectively.

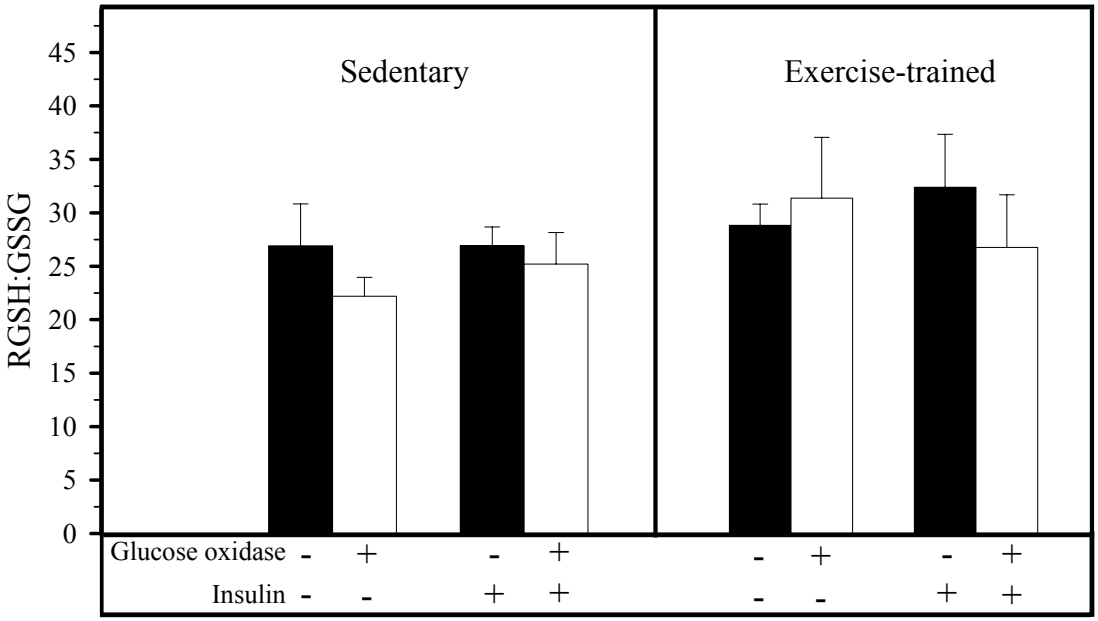

Figure 12. Reduced to oxidized glutathione ratio of incubated soleus muscle of the sedentary control and exercise-trained groups in the absence and presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means ± SEM; n = 9 in each group.

The contents of TGSH, RGSH, and GSSG in the incubated EDL muscles from the sedentary and exercise-trained animals are shown in Figures 13, 14, and 15, respectively. Oxidative stress significantly reduced the contents of TGSH and RGSH in the EDL muscles derived from both the sedentary and the exercise-trained animals. The GSSG contents in the muscles of the control group were decreased by oxidative stress although a significant difference could be seen in the muscle that was incubated in the presence of H_2O_2 alone. In the muscles of the animals that underwent exercise training, the contents of GSSG were significantly declined in the presence of oxidative stress. In the control group, the TGSH contents in EDL muscle incubated in the four experimental conditions were 1.05±0.07, 0.63±0.05, 0.98±0.06, and 0.65±0.08 μmol/g muscle wet weight, respectively. The values of RGSH contents in EDL muscle incubated in the experimental conditions were 1.00±0.07, 0.60±0.05, 0.94±0.06, and 0.61±0.08 μmol/g muscle wet weight, respectively. For GSSG contents in the incubated EDL muscle, the values were 0.055±0.008, 0.035±0.004, 0.044±0.005, and 0.039±0.004 μmol/g muscle wet weight, respectively.

In exercise-trained group, the TGSH contents of the incubated EDL muscle were 1.02±0.05, 0.80±0.07, 1.02±0.05, and 0.77±0.06 μmol/g muscle wet weight, respectively. RGSH contents in EDL muscle were 0.97±0.05, 0.77±0.07, 0.97±0.05, and 0.73±0.06 μmol/g muscle wet weight, respectively. In addition, the GSSG contents in EDL muscle were 0.050±0.005, 0.034±0.005, 0.050±0.004, and 0.034±0.005 μmol/g muscle wet weight, respectively.

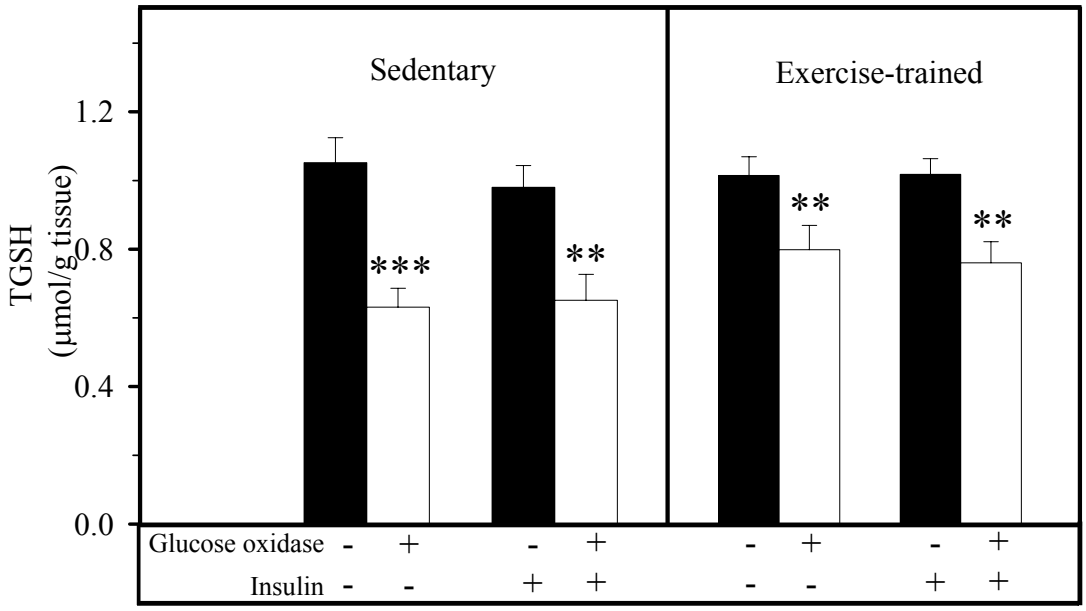

Figure 13. Total glutathione contents (µmol/g tissue) in incubated extensor digitorum longus muscle of rats in sedentary control and exercise-trained groups in the absence or presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means±SEM. **p<0.01, ***p<0.001 absence vs. presence of glucose oxidase. n = 10 in sedentary control group and n = 12 in exercise-trained group.

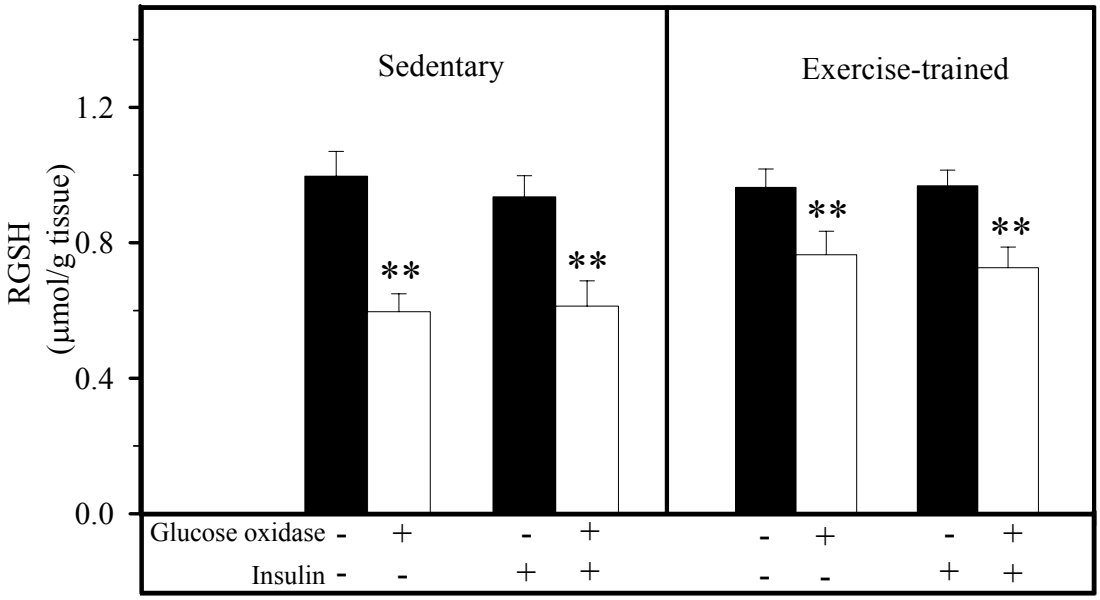

Figure 14. Reduced glutathione contents (µmol/g tissue) in incubated extensor digitorum longus muscle of rats in sedentary control and exercise-trained groups in the absence and presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means ± SEM.** $p<0.01$ absence vs. presence of glucose oxidase. n =10 in sedentary control group and n = 12 in exercise-trained group.

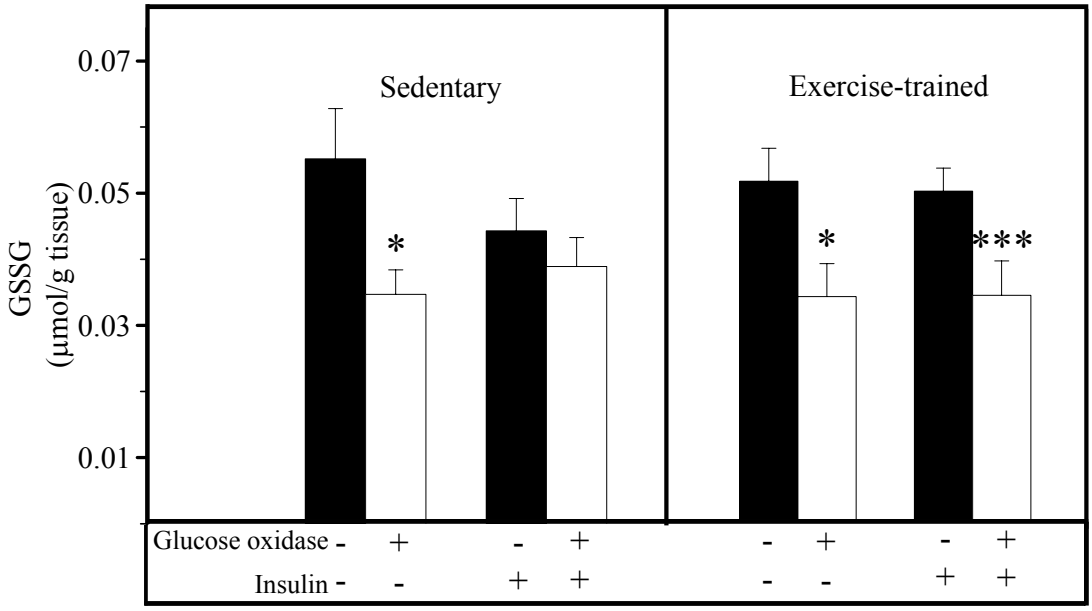

Figure 15. Oxidized glutathione contents (μmol/g tissue) in incubated extensor digitorum longus muscle of rats in sedentary control and exercise-trained groups in the absence or presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means ± SEM. * $p<0.05$, *** $p<0.001$ absence vs. presence of glucose oxidase. n =10 in sedentary control group and n =12 in exercise-trained group.

For the ratio of RGSH to GSSG in the incubated EDL muscle, the existence of oxidative stress tended to reduce, though not statistically significant, the glutathione redox status of the sedentary control group (Figure 16, left panel). The ratios of RGSH/GSSG in the incubated EDL muscle were 21.1±3.2, 18.5±2.2, 24.3±3.8, and 19.3±5.0, respectively. Even though the glutathione redox status in the EDL from the exercise-trained animals was resistant to oxidative stress, the changes in the RGSH/GSSG ratios did not reach any statistical significance (Figure 16, right panel). The ratios of the RGSH to GSSG in the incubated EDL muscle were 22.2±4.0, 27.6±4.4, 20.5±1.8, and 26.5±4.6, respectively.

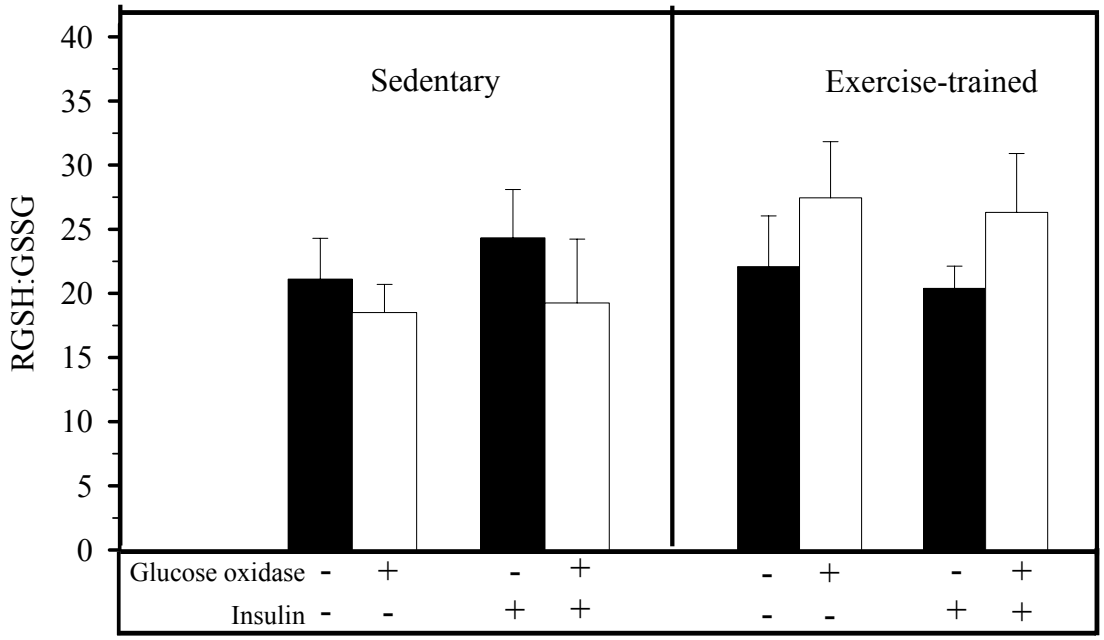

Figure 16. Reduced glutathione to oxidized glutathione ratio of incubated rat extensor digitorum longus muscle in sedentary control and exercise-trained groups in the absence and presence of glucose oxidase (100 mU/ml) and/or insulin (5 mU/ml) in the incubation medium. Values are means ± SEM. n =10 in sedentary control group and n =12 in exercise-trained group.

DISCUSSION

The present study determined the direct effect of oxidative stress on the insulin action on glucose transport activity in skeletal muscle of the insulin-sensitive Sprague-Dawley rats. The results indicated that oxidative stress in the absence of insulin had an insulin-like effect on muscle glucose uptake whereas the same oxidative stress negatively modulates insulin action on glucose transport activity in both type I slow-twitch and type II fast-twitch muscle tissues. More importantly, the present investigation evaluated the effectiveness of endurance exercise training on the protection against impaired insulin action in the skeletal muscle caused by oxidative stress. This study clearly showed that endurance exercise training at moderate-intensity is an effective intervention that can protect against oxidative stress-induced skeletal muscle insulin resistance in both slow-twitch and fast-twitch skeletal muscles.

The effect of oxidative stress (H_2O_2) on basal glucose transport activity in skeletal muscle was assessed. H_2O_2 by itself significantly activated basal glucose transport activity by 55% and 36% in the soleus and EDL, respectively. The result of the present study confirmed the finding from the study investigating the effects of exposing isolated skeletal muscle from normal lean Zucker rats to oxidative stress on the insulin signaling pathway and glucose transport activity (30). Exposed isolated soleus strips to oxidative stress caused 30% ($p<0.05$) increase in glucose transport activity, and enhancement of critical elements of the insulin signaling pathway was in part involved in the direct effect of oxidative stress on the stimulation of glucose transport activity in type I rat skeletal muscle. The latter observation was proven when the elevated glucose transport activity caused by oxidative stress was completely inhibited after the muscles were exposed to wortmannin – the phosphatidylinositol-3-kinase (PI3-kinase) inhibitor. Moreover, the oxidative stress brought about 468%, 87%, and 98% increases in tyrosine phosphorylation of the insulin receptor, IRS-1 associated with the p85 subunit of PI3-kinase, and in serine phosphorylation of Akt, respectively. In addition to the major insulin signaling elements activating glucose transport, the phosphorylation level of AMP-dependent kinase (AMPK) - an element in the contraction-dependent glucose transport pathway as well as stress-activated protein kinase, p38, were also increased by this oxidative stress by 108% and 45%, respectively. Collectively, the data taken from muscle tissues convincingly and surprisingly indicate that oxidative stress by itself exerts an insulin-like effect on glucose transport activity and insulin signaling molecules. However, this insulin-like action of oxidative stress on muscle glucose transport was not existent in muscle cell lines. Both acute (1 hr) and prolonged (18 hr)

exposures of rat L6 muscle cells to H_2O_2 did not enhance basal glucose transport (31,32). On the basis of applicability to man, the more physiological condition as in mammalian tissue appears to be more realistic. Moreover, it is important to note that the stimulatory effect of oxidative stress on muscle glucose transport rate observed in the present investigation possibly suggests the opportunity that oxidative stress is one of the signaling molecules involved in enhanced glucose transport activity under acute stress condition such as elevated glucose uptake during acute bout of muscle contraction.

Furthermore, the effect of oxidative stress on basal glucose transport activity in the muscle tissues from the exercise-trained animals was assessed. In order to determine an effectiveness of the exercise training protocol used in this study on the induction in physiological adaptation, heart weight to body weight ratio (an index of cardiac hypertrophy) was examined to represent systemic adaptations and oxidative enzyme - citrate synthase - was analyzed so as to indicate metabolic adaptations in skeletal muscle. The results showed that the exercise-trained animals possessed cardiac hypertrophy and higher level of oxidative enzyme activity in skeletal muscle. These data ascertain that the exercise training protocol was effective to result in systemic as well as skeletal muscle adaptations. Consistently with the findings in the sedentary control group, oxidative stress significantly activated basal glucose transport activity in soleus and EDL muscles from exercise-trained animals.

Even though the role of oxidative stress-related processes contributing to the metabolic abnormalities in the insulin-resistant state and diabetes was controversial, a connection between oxidative stress and such pathological conditions has been supported by a number of clinical and epidemiological studies (31,33-35). A cause and effect relationship between oxidative stress and insulin resistance was demonstrated for the first time in 1997 by Rudich *et al*. (14). After 18-hour exposure of 3T3-L1 adipocytes to oxidative stress, generated by micromolar concentrations of H_2O_2, an impaired insulin action on glucose transport was observed and was concomitant with increase in GLUT1 mRNA and protein, and reduction in GLUT4 mRNA and protein. Whereas these results clearly indicated that oxidative stress can directly impair the insulin action on glucose transport in insulin-sensitive cells, these observations were obtained from cell lines and it remains unknown whether the same responses are true in the insulin-sensitive tissues.

The direct effect of oxidative stress on insulin-stimulated glucose transport activity in mammalian skeletal muscle was reported for the first time by Saengsirisuwan *et al.* in 2003 (15). In contrast to the finding aforementioned by the present investigation and others (14,15,30)

that oxidative stress stimulated ($p<0.05$) basal glucose transport activity, the same oxidative stress significantly inhibited glucose transport activity in the presence of insulin by 28% in the soleus muscle isolated from the insulin-sensitive female lean Zucker rats. The observations in the present study that stimulatory effects of insulin on glucose transport activity in the soleus and EDL muscle from the male Sprague-Dawley rats were significantly inhibited by H_2O_2 support the direct role of oxidative stress in the etiology of skeletal muscle insulin resistance as demonstrated earlier in the muscle tissues by Saengsirisuwan *et al*. (15) as well as in cell lines by others (14,31,32,36). Furthermore, information obtained from the present investigation and Saengsirisuwan *et al*. (2003) together suggest that the inhibitory effects of oxidative stress on insulin-stimulated glucose transport activity in skeletal muscles are neither sex-specific nor specie-specific in the rodents.

The mechanisms by which oxidative stress results in impaired insulin action in skeletal muscle are of importance in understanding the pathophysiology of insulin-resistant condition and diabetes. Although it has not been clearly shown the mechanisms that oxidative stress brings about insulin resistance in skeletal muscle, a number of studies (36-38) using cell lines indicate that the stimulatory effects of insulin on the insulin signaling elements are interfered by oxidative stress. Many studies revealed that stress inducers, including H_2O_2, stimulate a number of stress-sensitive serine/threonine kinase cascades (39-41). The insulin signaling molecules such as insulin receptor and IRS proteins appear to be substrates for these activated serine kinases (35). Increased serine or threonine phosphorylation of insulin receptor substrates leads to a reduction in the extent of their tyrosine phosphorylation which attenuated insulin action (42-48). In addition, a study using rat L6 cells found that acute exposure of L6 cells to H_2O_2 activated p38 MAPK (one of the stress-sensitive serine/threonine kinase cascades) which was concurrent with inhibited insulin action and these effects of H_2O_2 were antagonized by p38 MAPK inhibitors (49). The study using 3T3-L1 adipocytes reported that activation of the proximal steps of the insulin-signaling cascade including insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation were suppressed by H_2O_2 (36,38).

Since an intact and dynamic actin cytoskeleton is required for insulin-mediated GLUT4 translocation and glucose uptake (50,51), any disruption in structure and function of the cytoskeleton will interfere with insulin-regulated translocation of GLUT4. A study in which actin dynamics was interfered by drugs such as cytochalasin D, latrunculin B or jasplakinolide showed that the drugs notably reduced not only actin remodeling but also insulin-stimulated GLUT4 translocation and glucose uptake (52,53). In muscle cells, actin filament remodeling

requires PI3-Kinase but not Akt activation (54). Nevertheless, the full activation of Akt is necessary for actin dynamics (53). This infers that Akt activation is downstream of actin remodeling. Moreover, it was found that insulin action on actin remodeling was inhibited under the presence of oxidative stress as well as under the high glucose and insulin condition (50).

As shown in Figures 1 and 2, the key finding from the present investigation is the effectiveness of exercise training to protect against insulin resistance caused by oxidative stress in both type I and II muscles. Consequently, in order to explore potential mechanism responsible for this beneficial effect of exercise training, the activities of non-enzymatic and enzymatic antioxidant systems were evaluated. The activities of the non-enzymatic antioxidant system is reflected by glutathione redox status or the ratio of reduced to oxidized glutathione (RGSH:GSSG) while the activities of glutathione peroxidase (GPX) and catalase (CAT) represented the activities of the enzymatic antioxidant system.

Glutathione redox status in the soleus and EDL muscles from the sedentary control animal was not altered following a direct exposure to oxidative stress. Therefore, it is likely that the stimulatory effect of oxidative stress on basal glucose transport and the inhibitory effect of oxidative stress on insulin-stimulated muscle glucose transport were due to the disturbance in the redox status. The redox status in the soleus and EDL muscles from the exercise-trained animals was also not affected by oxidative stress. Although TGSH and RGSH in EDL from both the control and the exercise-trained groups were significantly reduced following H_2O_2 incubation, such changes were proportional so that the redox status remained unchanged. This was partly the result of a concomitant decrease ($P<0.05$) in GSSG content. An unaltered glutathione redox status in soleus and DVL muscles in response to exercise training was previously reported by Leeuwenburgh *et al*. (55). Even though 10 wk of treadmill training (25 m/min, 10% grade for 2h/day, 5 days/wk) enhanced the activity of oxidative enzyme; citrate synthase, in DVL and soleus muscles of female Sprague-Dawley rats by 79% and 39% respectively, the ratio of RGSH to GSSG in both muscle types was not changed after training. A decrease in RGSH content in the soleus muscle by 14% ($p<0.01$) was observed instead. It is noteworthy that adaptation of RGSH to endurance training appears to be fiber-type specific. In contrast to type I slow-oxidative soleus muscle, an increase in RGSH was observed in type IIa muscle fibers like deep vastus lateralis (20,55). However, the present study did not find a rise in RGSH in type IIb EDL muscle. It might be a subfiber-type specific in the adaptation of RGSH content in skeletal muscle. The muscle fiber-type variation in exercise training-induced adaptation could be described by the rate of RGSH utilization and the capacity of RGSH uptake within different fiber

types. For instance, the RGSH-oxidizing rate in soleus muscle may overdo the capacity to import RGSH from an extracellular source, causing a net fall in the intramuscular RGSH content after training. Leeuwenburgh *et al*. showed that the membrane bound Υ-glutamyltranspeptidase (GGT; important for RGSH uptake) and the cytosolic Υ-glutamylcysteine synthetase (GCS; playing role in GSH synthesis) in the soleus muscle are 60%-70% and 32% lower, respectively, than those in DVL (55). Furthermore, various exercise-trained hindlimb muscles in dogs possessed an increase in RGSH content with increased Υ-glutamyl enzymes such as GGT and GCS (20,56,57). Accordingly, the present investigation showed a trend towards a lower TGSH and RGSH contents in the muscle from the exercise-trained animal compared with the sedentary control. Conclusively, the protective effect of exercise training on skeletal muscle insulin resistance caused by oxidative stress was not accounted for by an altered redox status.

To assess the activities of the enzymatic antioxidant system, two major muscular antioxidant enzymes, GPX and CAT, were investigated as their functions directly involve in the elimination of H_2O_2, an influential oxidative stress used in the current experiment. Even though the effects of exercise training on the enzymatic antioxidant system are still controversial, there are studies (19,20) supporting the notion that exercise training can enhance antioxidant enzyme activity as prolonged muscular exertion continuously stimulates production of reactive oxygen species and other oxidative stress which consequently leads to adaptation of the antioxidant defense system. GPX adaptation to exercise training is believed to be a muscle fiber-specific pattern. Type IIa muscle has been reported to be the most responsive to training (58), whereas type I and IIb muscle were not influenced by exercise training. Powers *et al*., for instance, showed that GPX activity in red gastrocnemius muscle (type IIa) of female Sprague-Dawley rats increased by 45% following endurance training (4 days/wk on a motor-driven treadmill for 10 wk) whereas the GPX activity was not changed in soleus (type I) and white gastrocnemius muscles (type IIb) (59). In addition, Leeuwenburgh *et al*. observed that GPX activity in deep vastus lateralis (DVL; type IIa) muscle was increased by 62% in response to 10 wk of treadmill training while the GPX activity in soleus and myocardium was not affected (55). Exercise training-induced adaptation in muscle CAT activity is controversial. Many studies reported no change in muscle CAT with training (60-62). However, some investigations (63-66) showed an increase in muscular CAT activity after training. In contrast, a few other studies even reported a reduced CAT activity by exercise training (67,68). As shown in Figures 5 and 6, the 6-wk treadmill running in rats using in the present study did not result in adaptation in the activities of GPX or CAT in both types of muscle, indicating that the beneficial effect of exercise training in

protecting against oxidative stress-induced skeletal muscle insulin resistance could not be explained by an adaptation of the enzymatic antioxidant system. Taken together, this investigation suggested that endurance exercise training did not lead to an adaptation in the oxidant status or the antioxidant enzyme activities. Therefore, the protective effects of endurance exercise training on impaired insulin action in skeletal muscle subjected to oxidative stress are independent on muscular antioxidant defense systems.

Although the precise underlying mechanisms responsible for this protective effect are currently unknown, information from studies using cell lines suggests that oxidative stress interferes activation of molecules in the insulin signaling pathway. The key molecules in the insulin signaling cascade including IRS-1 and Akt as well as common stress-activated signaling pathway such as nuclear factor-kB, p38 MAPK, and NH_2-terminal Jun kinases/stress-activated protein kinases are the appealing candidates for identifying how oxidative stress abrupt insulin action on glucose transport activity as well as for disclosing how exercise training exerts protection against oxidative stress-mediated skeletal muscle insulin resistance. The results from the present study have brought benefits to academic progress and human being. They specifically explained the causal role of oxidative stress in the occurrence of skeletal muscle insulin resistance. Additionally, the findings from this study point out the use of exercise training as an intervention for protection the development of skeletal muscle insulin resistance caused by oxidative stress and strengthen the notion that regular exercise at moderate exercise intensity is an effective tool for prevention a progress of insulin-resistant stage and subsequently type 2 diabetes mellitus.

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Output จากโครงการที่ได้รับทุนจาก สกอ. และ สกว.

1. อยู่ระหว่างดำเนินการเตรียมส่งผลงานเพื่อตีพิมพ์ในวารสารวิชาการนานาชาติ เรื่อง **Protection by exercise training against oxidant stress-induced skeletal muscle insulin resistance**
2. เสนอผลงานในที่ประชุมวิชาการ 2 ครั้ง
    a. V. Saengsirisuwan, K. Vichaiwong, C. Toskulkao.
    "Effect of Exercise Training on Insulin Action in Rat Skeletal Muscle Subjected to Oxidative Stress" ในการประชุมวิชาการสรีรวิทยาสมาคมแห่งประเทศไทย ครั้งที่ 34 วันที่ 24-27 เมษายน 2548 ณ ชุมพรคาบาน่ารีสอร์ท จ.ชุมพร
    b. K. Vichaiwong, C. Toskulkao, V. Saengsirisuwan
    "Prior Exercise Training Protects Against Oxidative Stress-Induced Skeletal Muscle Insulin Resistance" ในการประชุม 1st Asian Indoor Games International Scientific Congress ณ โรงแรมเอเชีย กรุงเทพฯ ระหว่างวันที่ 17-19 ตุลาคม 2548
    c. V. Saengsirisuwan, K. Vichaiwong, E.J. Henriksen, C. Toskulkao
    "Protection Against Oxidative Stress-Induced Skeletal Muscle Insulin Resistance by Exercise Training" ในการประชุมเพื่อเสนอผลงานวิจัยของนักวิจัยรุ่นใหม่และพบกับเมธีวิจัยอาวุโส สกว. โรงแรมรีเจนท์ ชะอำ ระหว่างวันที่ 13-15 ตุลาคม 2548
3. นำผลการศึกษาใช้ในเชิงวิชาการ โดยใช้เป็นข้อมูลในการสอนรายวิชา **SCPS 627 Current Topics in Cell Physiology** ซึ่งเป็นรายวิชาระดับบัณฑิตศึกษาของภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล ปีการศึกษา 2548-2549