

# Blood Lipid Peroxides and Muscle Damage Increased following Intensive Resistance Training of Female Weightlifters

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**ABSTRACT:** The aim of this study was to examine changes in muscle cell injury and antioxidant capacity of weightlifters following a 1-week intensive resistance-training regimen. Thirty-six female subjects participated in this study, and their ages ranged from 18 to 25 years. The sample group included 19 elite weightlifters with more than 3 years of weightlifting training experience, while the control group comprised 17 non-athletic individuals. Compared with non-athletes, weightlifters had significantly lower glutathione peroxidase activity and plasma vitamin C concentrations. Weightlifters also had significantly higher malondialdehyde + 4-hydroxy 2-(E)-nonenal (MDA+4-HNE) and thiobarbituric acid–reactive substance (TBARS) levels and creatine kinase (CK) activity. For weightlifters, the plasma vitamin E level and the activity of superoxide dismutase (SOD) decreased, and CK activity increased significantly ( $P < 0.05$ ) after a 1-week intensive resistance-training regimen. Both the TBARS levels and CK activity returned to values of pre-intensive training after a 2-day rest. The MDA+4-HNE level strongly correlated with CK activity in weightlifters ( $P < 0.05$ ). In conclusion, both long-term exercise training and 1 week of intensive resistance training resulted in increased oxidative stress and cell injury in female weightlifters. Furthermore, proper rest after intensive training was found to be important for recovery.

**KEYWORDS:** weightlifters; resistance training; lipid peroxidation; cellular damage

## INTRODUCTION

There is no doubt that exercise has many health benefits. Exercise is recommended for the prevention and management of many chronic diseases and for the maintenance of optimal health. However, performing strenuous physical activity can increase oxygen consumption by up to 10- to 15-fold over resting levels in order to meet energy demands. Exercise increases metabolism, and metabolic leakage from mitochondrial electron transport chains is now considered to be the most important source of oxygen-derived radicals. Increased oxygen uptake during exercise is accompanied by an elevation in reactive oxygen species (ROS), which is considered to be oxidative stress.<sup>1</sup> Oxidative stress has been linked to fatigue of skeletal and respiratory muscles, impaired performance and recovery, and the etiology of various diseases.<sup>2-4</sup>

Different athletes follow different training programs. Resistance training, also called strength training or weight training, is reported to have many benefits, such as weight control, prevention of osteoporosis, improvement of cardiovascular risk factors, and injury prevention.<sup>5,6</sup> Resistance training is a regular and important training program for weightlifters and power athletes, not only for improving the strength of muscles but also for maintaining a proper body composition.<sup>7,8</sup> However, an improper resistance-training program may increase cellular damage and oxidative stress in athletes. During resistance exercise, ischemia-reperfusion of muscles and production of free radicals via oxidative bursts from neutrophils occur and are considered serious.<sup>9</sup> The purpose of this study was to investigate cellular damage, the degree of oxidative stress, and antioxidative status both with habitual training and after a 1-week intensive resistance-training program.

## MATERIALS AND METHODS

### *Human Subjects*

The study was conducted at the National College of Physical Education and Sports, Taoyuan, Taiwan. Nineteen female elite weightlifters and 17 age-matched female non-athletes were recruited for this experiment, which was approved by the Taipei Medical University Subjects Committee. All weightlifters were members of the varsity team and participated regularly in national competitions. All weightlifters had undergone exercise training for more than 3 years. All subjects were free-living, and asked to maintain their usual food intake. The purpose and possible discomfort of the study were fully explained to each subject. All subjects signed informed consent forms. In a pre-study interview, each subject was asked to stop taking any vitamin and nutritional supplements 2 weeks before the study began.

### *Experimental Design*

The resistance-training program was designed by a nationally certified senior weightlifting coach for each athlete, and all weightlifters were asked to record their training program. All athletes had similar training regimens. The high-intensity

resistance-training lasted for 1 week, including 6 days, for two sessions per day, for 2–3 h per session. Each subject performed adequate warm-up sets. After the warm-up sets were completed, the resistance-training program was designed to increase the strength of all major muscle groups. A session consisted of the following: 3 sets of 3 repetitions on the shoulder press; 3 sets of 5 repetitions on the back squat; 1 set of 2 repetitions on abdominal curls; 3 sets of 5 repetitions on the dead lift; 1 set of 2 repetitions on the snatch; and 4 sets of 5 repetitions on the good morning. Resistance was set at 80% of 1 repetition maximum (1-RM). To control for aerobic exercise effects, weightlifters were required to rest for 30 to 60 s between each set and for 2 to 3 min between each exercise. A 2-day rest followed the 1-week high-intensity resistance training.

### *Sample Collection and Analysis*

The total body fat and fat-free mass (FFM) of subjects were measured using a Biodynamics Body Composition Analyzer (Biodynamics Corp., Seattle, WA) according to a bioelectrical impedance analysis (BIA). Ten milliliters of blood were collected from each subject after 12 h of fasting, prior to and after the 1-week training, as well as from all weightlifters, after the 2-day rest that followed. Red blood cell portions of whole-blood samples were separated out for subsequent superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) analyses using commercial kits (Randox Laboratories Ltd., Crumlin, Antrim, UK). Both plasma vitamin C and vitamin E were analyzed using reverse-phase high-performance liquid chromatography (HPLC) with a UV detector at 254 nm, and a fluorescence detector with excitation at 292 nm and emission at 340 nm, respectively. The 4 × 250 mm and 4 × 125 mm Lichrosphere 100RP-18 column (Merck, Darmstadt, Germany), containing 5-mm particles protected by a guard column, were used for vitamin C and vitamin E analysis, respectively. The mobile phase for vitamin C contained 0.5 mM PICB (1-Pentane sulfuric acid sodium salt) adjusted to pH 3.1 with glacial acetic acid. Pure methanol was used as the mobile phase for vitamin E analysis. Plasma leptin was measured with an immunoradioassay using a commercial kit. The measurement of total antioxidant status (TAS) was based on the method of Miller *et al.*<sup>10</sup> using commercial kits. Plasma creatine kinase (CK) activity was determined in duplicate using a colorimetric assay method with a Johnson & Johnson DT-60 at 680 nm. Plasma lipid peroxidation was assessed by a modified spectrofluorometric measurement of thiobarbituric acid-reactive substances (TBARS). Plasma glutathione (GSH), malondialdehyde (MDA), and 4-hydroxy-2(E)-nonenal (4-HNE) were also directly determined using a commercial kit (Calibiochem-Novabiochem Co., San Diego, CA).

### *Statistical Analysis*

Values are expressed as the mean ± SD. Differences between weightlifters and non-athletes were compared using the independent-sample *t*-test. Data obtained before and after the 1-week training, and after the 2-day rest, were compared using Student's paired *t*-test with the Statistical Analysis System (SAS) program. Differences of *P* < 0.05 were considered significant.

**TABLE 1. Characteristics of weightlifters and non-athletes<sup>a</sup>**

Characteristics	Weightlifters (n = 19)	Non-athletes (n = 17)
Age (years)	20.7 ± 1.6	21.9 ± 0.9
Height (cm)	156.8 ± 6.9	160.8 ± 5.2
Weight (kg)	67.9 ± 7.1*	52.7 ± 7.0
Body mass index (kg/m <sup>2</sup> )	27.6 ± 8.1*	20.3 ± 5.1
Body fat (%)	23.6 ± 3.4	22.0 ± 3.8
(kg)	14.9 ± 3.7*	11.7 ± 2.2
Fat-free mass (%)	76.4 ± 3.4	77.3 ± 4.3
(kg)	47.6 ± 7.1*	41.4 ± 6.3
Arm circumference (cm)	12.2 ± 1.2*	10.6 ± 1.0
Weightlifting history (years)	5.3 ± 1.8	—

<sup>a</sup>Values are the mean ± SD.

\*Significantly different from the non-athlete group by independent-sample *t*-test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

The characteristics of subjects are shown in TABLE 1. Both groups were similar in age and height. The body weight, body mass index (BMI), and arm circumference of weightlifters were higher than those of non-athletes. There were no significant differences in body fat (%) or FFM (%) between the two groups; however, weightlifters had a higher amount of both body fat (kg) and FFM (kg) than did non-athletes. Because most weightlifters in this study had more than 3 years of weightlifting history and regular exercise training and weightlifting experience, they had a different body composition from that of the non-athletes. The data reported are similar to those of previous studies.<sup>11,12</sup>

The biochemical parameters of weightlifters and non-athletes are presented in TABLE 2. Compared with non-athletes, weightlifters had higher plasma levels of vitamin E and GSH, and lower vitamin C and GSH-Px activity than did non-athletes. However, the creatine kinase (CK) activity and TBARS levels in weightlifters were significantly higher than those of the control group. Long-term regular training fostered a worse antioxidative status and more serious cellular damage in weightlifters compared with non-athletes.

TABLE 3 presents changes in biochemical parameters after the 1 week of intensive training and after a 2-day rest. The level of plasma vitamin E showed a significant 51% decrease after 1 week of high-intensity training, but increased after the 2-day rest. The same was true for SOD, which showed a significant 14% activity decrease after 1 week of high-intensity training. Levels of vitamin C and TAS and the activity of GSH-Px decreased after 1 week of high-intensity training; however, there were no significant differences between pre-training levels and those seen after the 2-day rest, respectively.

As seen in TABLE 4, after weight training, CK activity significantly increased, -by 95.4%, after 1 week of high-intensity training, but significantly decreased to a level similar to that of pre-training after a 2-day rest. After 1 week of high-intensity train-

**TABLE 2. Levels of plasma biochemical parameters of weightlifters and non-athletes<sup>a</sup>**

Plasma Biochemical Parameters	Weightlifters (n = 19)	Non-athletes (n = 17)
Leptin (ng/mL)	11.70 ± 4.85	10.8 ± 2.94
Vitamin C (mol/L)	38.20 ± 17.02*	49.15 ± 13.81
Vitamin E (mol/L)	23.93 ± 10.27*	20.85 ± 8.26
Glutathione (µmol/L)	287.34 ± 81.34*	198.34 ± 46.32
TAS (mmol/L)	1.54 ± 0.29	1.15 ± 0.21
SOD (U/mg Hb)	18.20 ± 2.59	17.46 ± 2.65
GSH-Px (U/g Hb)	26.25 ± 11.57*	38.07 ± 6.02
Creatine kinase (U/L)	117.01 ± 45.72*	80.85 ± 14.14
MDA+4-HNE (µmol/L)	78.87 ± 17.24	72.42 ± 17.11
TBARS (µmol/L)	5.82 ± 1.96*	3.89 ± 0.78

<sup>a</sup>Values are the mean ± SD.

\*Significantly different from the non-athlete group by independent-sample *t*-test ( $P < 0.05$ ).

ABBREVIATIONS: TAS, total antioxidant status; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA+4-HNE, malondialdehyde + 4-hydroxy 2-(E)-nonenal; TBARS, thiobarbituric acid-reactive substances.

**TABLE 3. Levels of blood antioxidant substances and activities of antioxidant enzymes of weightlifters before and after 1 week of resistance training and after a 2-day rest<sup>a</sup>**

	Pre-training (n = 19)	Post-training (n = 19)	After a 2-day rest (n = 17)
Vitamin C (µmol/L)	38.20 ± 17.02	33.30 ± 10.32	36.14 ± 7.25
Vitamin E (µmol/L)	23.93 ± 10.27	11.35 ± 5.29*	17.11 ± 6.18
Glutathione (µmol/L)	287.34 ± 81.34	301.85 ± 65.8	283.35 ± 42.64
TAS (mmol/L)	1.54 ± 0.29	1.28 ± 0.57	1.29 ± 0.47
SOD (U/mg Hb)	18.20 ± 2.59	15.64 ± 2.89*	16.09 ± 2.47
GSH-Px (U/g Hb)	26.25 ± 11.57	20.54 ± 7.42	25.29 ± 8.96

<sup>a</sup>Values are the mean ± SD.

\*Significantly different from pre-training by Student's paired *t*-test ( $P < 0.05$ ).

ABBREVIATIONS: TAS, total antioxidant status; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase.

**TABLE 4. Activities of creatine kinase and levels of lipid peroxidation of weightlifters before and after 1 week of resistance training, and after a 2-day rest<sup>a</sup>**

	Pre-training (n = 19)	Post-training (n = 19)	After a 2-day rest (n = 17)
Creatine kinase (U/L)	117.01 ± 45.72	220.60 ± 109.52*	118.84 ± 42.25 <sup>#</sup>
MDA+4-HNE (µmol/L)	78.87 ± 17.24	101.75 ± 20.29*	100.38 ± 20.58
TBARS (µmol/L)	5.82 ± 1.96	7.23 ± 1.90*	5.09 ± 2.14 <sup>#</sup>

<sup>a</sup>Values are the mean ± SD.

\*Significantly different from pre-training by Student's paired *t*-test ( $P < 0.05$ ).

<sup>#</sup>Significantly different from post-training by Student's paired *t*-test ( $P < 0.05$ ).

ing, there were significant increases in the indices of lipid peroxidation of 29% for MDA+4-HNE, and 21% for TBARS. Similar to CK activity, the levels of TBARS significantly decreased after the 2-day rest. A significant positive correlation between plasma CK activity and MDA+4-HNE levels was found ( $r = 0.38$ ,  $P < 0.05$ ). Both MDA+4-HNE and TBARS are usually used as quantitative markers for free radical interactions with cell membranes.<sup>13</sup>

In this study, the increased activity of the cytoplasmic enzyme CK indicated that tissue damage had occurred, especially in muscles.<sup>4</sup> The CK, MDA+4-HNE, and TBARS responses agreed with those of some previous investigations involving aerobic-type exercise.<sup>14</sup> It is unclear, however, why both resistance exercise and aerobic-type exercise result in similar increases in free radical production. It might be expected that the mechanisms for the increase in free radical formation during resistance exercise and aerobic-type exercise would differ.

Resistance training consists of repetitive, static muscle actions. These include concentric and eccentric muscle actions, which are considered to be, respectively, a low- or high-intensity resistance exercise protocol. Based on previous investigations, it was determined that the intensity of the exercise protocol used is a primary factor in creating a physiological environment for increased free radical production.<sup>15,16</sup> Plasma MDA was also found to be elevated after heavy resistance exercise involving upper and lower body muscles in recreational weight-training exercises.<sup>13</sup>

As previously mentioned, hyperoxia at the site of mitochondria may be a more prominent mechanism for free radical formation during aerobic-type exercise.<sup>17,18</sup> In contrast, intense muscle contractions associated with resistance exercise may result in ischemia-reperfusion at the site of the active muscles. The free radicals act as a mediator of ischemia-reperfusion injury to skeletal muscles and result in muscle injury accompanied by increased amounts of CK. Kanter *et al.*<sup>19,20</sup> have also shown that plasma MDA measurements correlate with CK activity during exercise. It is now clear that high-intensity whole-body resistance exercise can result in the formation of free radicals. These free radicals may play a role in how muscle tissues adapt to the physiological stress caused by resistance exercise. Ischemia-reperfusion during resistance exercise at the site of the muscle, and post-exercise production of free radicals via oxidative bursts from neutrophils, are realistic concepts that must be seriously considered.<sup>9</sup>

In conclusion, this study demonstrates that both regular long-term exercise training and 1-week intensive resistance training can result in increased oxidative stress and cell injury in female weightlifters. Further, proper rest after intensive training is necessary in order to reduce exercise-induced oxidative damage and allow the athletes to recover. The results obtained from this study may provide coaches and sports specialists with a foundation and reference information from which to design proper training programs for weightlifters, power athletes, and muscle builders in the future.

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