Comparison between Glucose-6-Phosphate Dehydrogenase–Deficient and Normal Individuals after Eccentric Exercise

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ABSTRACT

THEODOROU, A. A., M. G. NIKOLAIDIS, V. PASCHALIS, G. K. SAKELLARIOU, I. G. FATOUROS, Y. KOUTEDAKIS, and A. Z. JAMURTAS. Comparison between Glucose-6-Phosphate Dehydrogenase–Deficient and Normal Individuals after Eccentric Exercise. *Med. Sci. Sports Exerc.*, Vol. 42, No. 6, pp. 1113–1121, 2010. **Purpose**: Theoretically, glucose-6-phosphate dehydrogenase (G6PD)-deficient compared with nondeficient individuals may be less capable of performing physical activities and/or may be more vulnerable to muscle damage and oxidative stress. Therefore, the purpose of the present study was to examine the effects of a resistance muscle-damaging exercise bout on muscle function and damage, redox status in plasma, and erythrocytes and hemolysis. **Methods**: Nine males with established G6PD deficiency and nine males with normal G6PD activity performed an eccentric muscle-damaging exercise protocol. Isometric torque, range of motion, delayed onset muscle soreness, and creatine kinase were measured as indices of muscle function and damage. Reduced glutathione, oxidized glutathione, thiobarbituric acid–reactive substances, protein carbonyls, catalase, uric acid, and total antioxidant capacity were measured as indices of blood redox status. Plasma hemoglobin and bilirubin were measured as indices of hemolysis. All measurements conducted before, immediately after, and 1, 2, 3, 4, and 5 d after exercise. **Results**: All indices measured confirmed that eccentric exercise induced severe muscle damage, oxidative stress, and hemolysis, peaking at 2 and 3 d postexercise. Lower resting levels of reduced glutathione were detected in the G6PD-deficient group compared with the control group. Nevertheless, both the time course and the magnitude of the changes of the selected muscle performance, redox status (both in plasma and in erythrocytes), and hemolysis indices measured were similar between the two groups. **Conclusions**: The present study indicates that G6PD-deficient individuals may participate in high-intensity muscle-damaging activities, without a negative impact on muscle function, blood redox status, and hemolysis. **Key Words:** FREE RADICALS, LIPID PEROXIDATION, HEMOLYSIS, MUSCLE DAMAGE

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common genetic disorders, affecting approximately 7% of the world population (24). G6PD catalyzes the first reaction of the pentose phosphate pathway involving the conversion of glucose into pentose sugars while providing reducing power in the form of nicotinamide adenine dinucleotide phosphate (NADPH) (24). The most affected cell from this defect is the erythrocyte. Erythrocytes lack mitochondria, and the pentose phosphate pathway is their only source of NADPH (24). Insufficient levels of NADPH lead to impaired regeneration of the reduced form of glutathione (GSH) from the oxidized form (GSSG) (24). Indeed, erythrocytes of G6PD-deficient individuals contain approximately half the level of GSH compared with nondeficient individuals (16,18,21,27). As a result, erythrocytes of G6PD-deficient individuals may be more vulnerable to oxidative damage and hemolysis (24,35). It is notable that oxidative damage and hemolysis may have important physiological repercussions, for instance, the decreased ability of erythrocytes to transfer oxygen to tissues (2).

The enzyme defect in G6PD-deficient individuals is present in both the erythrocyte and the muscle (29), and a positive relationship exists between the two in G6PD activity (29). It has been reported that G6PD activity is rapidly increased in response to oxidative stress in cardiomyocytes and that inhibition of G6PD depletes cytosolic GSH levels, resulting in cardiomyocyte contractile dysfunction

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(14). Furthermore, mice lacking G6PD exhibited severe heart contractile dysfunction, which is associated with depletion of total glutathione stores and impaired regeneration of GSH from its oxidized form (15). As far as skeletal muscle is concerned, supplementation with *N*-acetylcysteine (a drug supporting GSH synthesis) increased GSH levels and improved muscle performance (20,22,23,34,37). In addition, two case reports described severe rhabdomyolysis after exercise in G6PD-deficient individuals (5,30). Collectively, a question that emerges is whether G6PD-deficient individuals are less capable of performing physical activities and/or are more vulnerable to muscle damage. We are not aware of any studies that examined muscle function and performance in G6PD-deficient individual, because most of the relevant studies focused on the clinical manifestations of this enzyme defect using *in vitro* approaches (24,35).

On the basis of studies from our group, it seems that G6PD-deficient individuals are able to perform aerobic non–muscle-damaging exercise without experiencing greater oxidative stress in the blood compared with individuals with normal G6PD activity (18,27). In these studies, redox status indices were measured in plasma and whole blood and not in erythrocytes, which is the primary target for oxidative damage in G6PD-deficient individuals (35). Moreover, muscle function and performance were not monitored. Muscle-damaging exercise compared with non–muscle-damaging exercise is known to induce greater and more prolonged disturbances in muscle function and redox status, lasting up to 3–5 d during recovery (8,26,28). Therefore, the aims of the present study were to examine the effects of a resistance muscle-damaging exercise bout on (i) muscle damage and performance, (ii) redox status in plasma and erythrocytes, and (iii) hemolysis. We hypothesized that G6PD-deficient individuals will experience greater muscle damage and impaired performance and larger disturbances in redox homeostasis as well as larger hemolysis.

METHODS

Subjects. Subjects were recruited after advertising the study in the local media. The initial selection of the G6PD-deficient participants had been performed through self-reports from previous diagnosis. Moreover, to verify that indeed they were deficient, we measured G6PD activity in every participant. The entry criterion was G6PD activity >8.0 U·g Hb^{-1} for the control group and <1.0 U·g Hb^{-1} for the G6PD-deficient group, respectively. Nine males with established G6PD deficiency and nine males with normal G6PD activity (matched with the G6PD-deficient subjects for age and maximal isometric torque) participated in the present study. Subjects had no experience with muscle-damaging exercise for at least 6 months before the study and were not taking any medications, or dietary supplements. They were instructed to abstain from strenuous exercise for 7 d before and during data collection. A written informed consent to participate in the study was provided by all participants after the volunteers were informed about all risks, discomforts, and benefits involved in the study. The procedures were in accordance with the 1975 Declaration of Helsinki, and approval was received from the institutional review board.

Design. Volunteers performed an isokinetic eccentric contraction session of the knee extensors of both legs. Muscle damage and biochemical indices were determined before, immediately after, and at 1, 2, 3, 4, and 5 d postexercise. All measurements and blood samplings were performed between 9 and 11 a.m. after an overnight fast and abstaining from alcohol and caffeine for 24 h. Each subject was familiarized at least for 5 d before the experimental procedures. This familiarization procedure involved 8–10 isokinetic eccentric actions at very low intensity not capable of inducing muscle damage as well as assessment of range of motion (ROM) and delayed onset muscle soreness (DOMS).

Anthropometric measurements. Each participant reported to the laboratory in the morning. During their first visit, body mass was measured to the nearest 0.5 kg (Beam Balance 710; Seca, Birmingham, UK), with subjects lightly dressed and barefoot. Standing height was measured to the nearest 0.5 cm (Stadiometer 208, Seca). Percentage body fat was calculated from seven skinfold measures (average of two measurements of each site) using a Harpenden caliper (John Bull, St. Albans, UK). The Siri skinfold equation was used to calculate body fat.

Isokinetic exercise protocol. The isokinetic dynamometer Cybex Norm (Cybex, Ronkonkoma, NY) was calibrated weekly according to the manufacturer's instructions. Subjects were seated (120° hip angle) with the lateral femoral condyle aligned with the axis of rotation of the dynamometer and were coupled to the dynamometer by an ankle cuff attached proximal to the lateral malleolus. The position of each subject was recorded and used in follow-up measurements. Each subject's functional ROM was set electronically between full extension (0°) and 120° of knee flexion to prevent hyperextension and hyperflexion. Gravitational corrections were made to account for the effect of limb weight on torque measurements. Feedback of the intensity and duration of eccentric exercise was provided automatically by the dynamometer. Subjects had to accomplish 5 sets of 15 eccentric maximal voluntary contractions with each leg at an angular velocity of $60°\cdot s^{-1}$ in the seated position. A 2-min rest interval was incorporated between sets. Before each exercise session, subjects performed a warm-up consisting of 8-min cycling on a Monark cycle ergometer (Monark, Vansbro, Sweden) at 70 rpm and 50 W followed by 5 min of ordinary stretching exercises of the major muscle groups of the lower limbs.

Muscle function and damage. The isokinetic dynamometer was also used for the measurement of isometric knee extensor peak torque at 90° knee flexion. The best of the three maximal voluntary contractions was recorded. To ensure that subjects provided their maximal effort, we

repeated measurements if the difference between the lower and the higher torque value exceeded 10%. There was a 2-min rest between isometric efforts. The test–retest reliability of isometric peak torque measurement was 0.97 as measured through the intraclass correlation coefficient test. The assessment of pain-free ROM was performed manually. The investigator moved the calf at a very low angular velocity from 0 knee extension to the position where the subject felt any discomfort. The test–retest reliability of ROM measurement was 0.95 as measured through the intraclass correlation coefficient test. Each subject assessed DOMS by palpation of the muscle belly in the distal region of the vastus medialis, vastus lateralis, and rectus femoris in a seated position with the muscles relaxed. The assessment of soreness of the exercised lower limb was also performed during walking. Perceived soreness for both conditions was rated on a scale ranging from 1 (*normal*) to 10 (*very, very sore*). The test–retest reliability of DOMS measurement by palpation and walking was 0.95 and 0.93, respectively, as measured through the intraclass correlation coefficient test.

Blood collection and handling. Before eccentric exercise, immediately after exercise, and at 1, 2, 3, 4, and 5 d postexercise, blood samples were drawn from a forearm vein. Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged immediately at 1370*g* for 10 min at 4°C, and the plasma was collected. The packed erythrocytes were lysed with 1:1 (v/v) distilled water, inverted vigorously, and centrifuged at 4000*g* for 15 min at 4°C. Another portion of blood (2 mL) was collected in EDTA tubes and was placed immediately on ice for the determination of G6PD activity, hematocrit, and hemoglobin.

Assays. For GSH, 20 μL of erythrocyte lysate treated with 5% TCA mixed with 660 μL of 67 mM sodium potassium phosphate (pH 8.0) and 330 μL of 1 mM 5,5′-dithiobis-2 nitrobenzoate. The samples were incubated in the dark at room temperature for 45 min, and the absorbance was read at 412 nm. GSSG was assayed by treating 50 μL of erythrocyte lysate with 5% TCA and neutralized up to pH 7.0–7.5 with NaOH. One microliter of 2-vinyl pyridine was added, and the samples were incubated for 2 h at room temperature. Five microliters of erythrocyte lysate treated with TCA was mixed with 600 μL of 143 mM sodium phosphate (6.3 mM of EDTA, pH 7.5), 100 μL of 3 mM NADPH, 100 μL of 10 mM 5,5′-dithiobis-2 nitrobenzoate, and 194 μL of distilled water. The samples were incubated for 10 min at room temperature. After the addition of 1 μL of glutathione reductase, the change in absorbance at 412 nm was read for 3 min. GSH and GSSG concentration was calculated by calibration curves constructed using commercial standards. The intraassay coefficient of variation for GSH was 4.2% and for GSSG was 3.6%.

For thiobarbituric acid–reactive substances (TBARS) determination, 100 μL of plasma or erythrocyte lysate was mixed with 500 μL of 35% TCA and 500 μL of Tris–HCl (200 mM, pH 7.4) and incubated for 10 min at room temperature. One milliliter of 2 M Na_2SO_4 and 55 mM thiobarbituric acid solution were added, and the samples were incubated at 95°C for 45 min. The samples were cooled on ice for 5 min and were vortexed after adding 1 mL of 70% TCA. The samples were centrifuged at 15,000*g* for 3 min, and the absorbance of the supernatant was read at 530 nm. A baseline absorbance was taken into account by running a blank along with all samples during the measurement. Calculation of TBARS concentration was based on the molar extinction coefficient of malondialdehyde. The intraassay coefficient of variation for TBARS measured in plasma was 6.5% and for TBARS in erythrocytes was 5.1%.

Protein carbonyls were determined adding 50 μL of 20% TCA to 50 μL of plasma or erythrocyte lysate (diluted 1:10). This mixture was incubated in an ice bath for 15 min and centrifuged at 15,000*g* for 5 min at 4°C. The supernatant was discarded, and 500 μL of 10 mM 2,4-dinitrophenylhydrazine (in 2.5 N HCL) for the sample or 500 μL of 2.5 N HCL for the blank was added in the pellet. The samples were incubated in the dark at room temperature for 1 h, with intermittent vortexing every 15 min, and were centrifuged at 15,000*g* for 5 min at 4°C. The supernatant was discarded, and 1 mL of 10% TCA was added, vortexed, and centrifuged at 15,000*g* for 5 min at 4°C. The supernatant was discarded, and 1 mL of ethanol–ethyl acetate (1:1 v/v) was added, vortexed, and centrifuged at 15,000*g* for 5 min at 4°C. This washing step was repeated twice. The supernatant was discarded, and 1 mL of 5 M urea (pH 2.3) was added, vortexed, and incubated at 37°C for 15 min. The samples were centrifuged at 15,000*g* for 3 min at 4°C, and the absorbance was read at 375 nm. Calculation of protein carbonyl concentration was based on the molar extinction coefficient of dinitrophenylhydrazine. The intraassay coefficient of variation for proteins carbonyls measured in plasma was 4.4% and for protein carbonyls in erythrocytes was 4.9%.

Catalase activity was determined adding 4 μL of plasma or erythrocyte lysate (diluted 1:10), respectively, to 2991 or 2955 μL of 67 mM sodium potassium phosphate (pH 7.4), and the samples were incubated at 37°C for 10 min. Five microliters of 30% hydrogen peroxide was added to the samples, and the change in absorbance was immediately read at 240 nm for 1.5 min. Calculation of catalase activity was based on the molar extinction coefficient of H_2O_2. The intraassay coefficient of variation for catalase measured in plasma was 5.3% and for catalase in erythrocytes was 3.8%.

Total antioxidant capacity (TAC) was determined adding 20 μL of plasma to 480 μL of 10 mM sodium potassium phosphate (pH 7.4) and 500 μL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, and the samples were incubated in the dark for 30 min at room temperature. The samples were centrifuged for 3 min at 20,000*g*, and the absorbance was read at 520 nm. TAC is presented as millimole of 1,1-diphenyl-2-picrylhydrazyl

TABLE 1. Anthropometric characteristics and hematological parameters of control and G6PD-deficient individuals (mean ± SEM).

	Control	G6PD Deficient
Age (yr)	22.6 ± 0.4	23.3 ± 0.8
Height (cm)	176.8 ± 1.9	178.6 ± 2.4
Weight (kg)	75.7 ± 1.9	77.1 ± 2.3
Body fat (%)	15.4 ± 0.8	16.1 ± 1.5
Hematocrit (%)	45.2 ± 0.9	41.5 ± 1.1*
Hemoglobin ($g \cdot dL^{-1}$)	15.3 ± 0.8	14.1 ± 0.9*
G6PD activity ($U \cdot g\ Hb^{-1}$)	9.94 ± 1.03	0.62 ± 0.12*

* Significantly different from the respective value in the control group.

radical (DPPH) reduced to 1,1-diphenyl-2-picrylhydrazine (DPPH:H) by the antioxidants of plasma. The intraassay coefficient of variation for TAC was 2.9%.

Creatine kinase (CK) activity was assayed using a kit from Spinreact (Sant Esteve, Spain). G6PD activity was determined using a kit from Sigma (St. Louis, MO) based on the spectrophotometric analysis of the rate of NADPH production from $NADP^+$ (6). Uric acid and biliburibin were determined using kits from Zafiropoulos (Athens, Greece). Plasma hemoglobin was assayed using a kit from Bio-Assays System (Hayward, CA). Hematocrit and hemoglobin were measured in a Sysmex K-1000 (TOA Electronics, Japan) autoanalyzer. The intraassay coefficient of variation for CK, G6PD, uric acid, bilirubin, plasma hemoglobin, hematocrit, and whole-blood hemoglobin was 3.2%, 2.9%, 3.4%, 2.5%, 2.1%, 1.8%, and 1.9%, respectively. Each assay was performed in duplicates and within 3 months of the blood collection. Blood samples were stored in multiple aliquots at –80°C and thawed only once before analysis.

Dietary analysis. Participants were asked to record their diet for 3 d before the exercise protocol and during the blood collection period. Diet records were analyzed using the nutritional analysis system Science Fit Diet 200A (Sciencefit, Greece).

Statistical analysis. Data are presented as mean ± SEM. The distribution of all dependent variables was examined by the Shapiro–Wilk test and was found not to differ significantly from normal. Two-way ANOVA (status × time) with repeated measurements on time were used to analyze isometric peak torque, DOMS, ROM, and all redox status indices. If a significant interaction was obtained, pairwise comparisons were performed through simple main effect analysis. Differences on physical characteristics, diet, and G6PD activity between the control and the G6PD-deficient group were examined by unpaired Student's *t*-test. The level of statistical significance was set at $\alpha = 0.05$. SPSS version 15.0 was used for all analyses (SPSS Inc., Chicago, IL).

TABLE 2. Analysis of daily energy intake of control and G6PD-deficient individuals (mean ± SEM).

	Control	G6PD Deficient
Energy (kcal)	2618 ± 106	2734 ± 150
Carbohydrate (% energy)	53.3 ± 2.7	51.7 ± 2.2
Fat (% energy)	31.2 ± 2.5	31.7 ± 3.0
Protein (% energy)	15.5 ± 0.8	16.6 ± 1.1
Vitamin A (mg, RE)	1.08 ± 0.14	1.08 ± 0.10
Vitamin C (mg)	128 ± 13	125 ± 14
Vitamin E (mg, α-TE)	8.2 ± 0.7	8.4 ± 0.6
Selenium (μg)	44.8 ± 3.0	47.2 ± 2.8

RE, retinol equivalents; α-TE, alpha-tocopherol equivalents.

RESULTS

Physical characteristics, hematology, G6PD activity, and dietary intake. There were no significant differences in physical characteristics between the two groups ($P > 0.05$; Table 1). Hematocrit and hemoglobin were significantly higher in the control group ($P < 0.05$; Table 1). G6PD activity was significantly higher (by 16 times; $P < 0.001$) in the control compared with the G6PD-deficient group (Table 1). No significant differences were found in daily energy, macronutrient, and micronutrient intake between the two groups during this period ($P > 0.05$; Table 2).

Muscle function and damage. There was no significant main effect of group or time × group interaction concerning any muscle damage index. Regarding isometric torque, there was a significant main effect of time ($P < 0.001$; Fig. 1A), with torque declining at all time

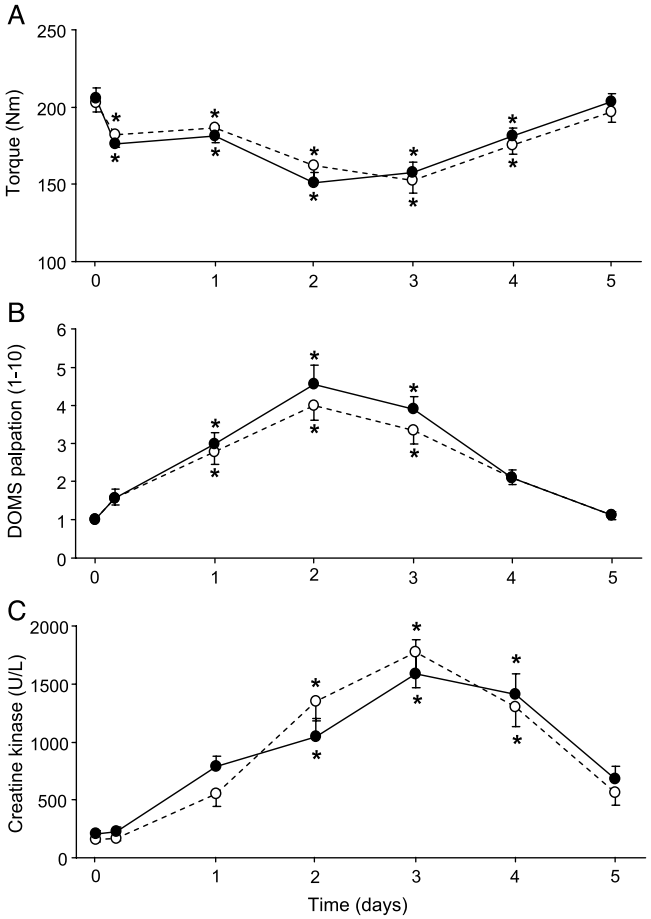

FIGURE 1—Isometric peak torque (A), DOMS after palpation (B), and CK (C) in the control (open circles) and G6PD-deficient individuals (closed circles) (mean ± SEM). *Significantly different from the preexercise value in the same group ($P < 0.05$). ROM, range of movement; DOMS, delayed onset muscle soreness; CK, creatine kinase.

points except 5 d after exercise in both groups. Concerning ROM, there was a significant main effect of time ($P < 0.001$), with ROM values peaking 3 d after exercise in both groups. Regarding DOMS, there was a significant main effect of time after palpation ($P < 0.001$; Fig. 1B) and after walking ($P < 0.001$) in both groups. Compared with baseline values, DOMS after walking was increased immediately after exercise and remained increased up to 4 d in both groups. Finally, regarding CK activity, there was a significant main effect of time ($P < 0.001$; Fig. 1C), with CK values being significantly higher at 2, 3, and 4 d after exercise in both groups.

Plasma redox status. There was no significant main effect of group or time × group interaction concerning plasma TBARS (Fig. 2A), protein carbonyls (Fig. 2C), catalase (Fig. 2E), uric acid (Fig. 2G), or TAC (Fig. 2H). However, there was a significant main effect of time ($P < 0.001$) for all plasma redox status indices, generally increasing at 2, 3, and 4 d after exercise in both groups compared with baseline values.

Erythrocyte redox status. There was no significant main effect of group or time × group interaction concerning erythrocytes TBARS (Fig. 2B), protein carbonyls (Fig. 2D), and catalase (Fig. 2F). However, there was a significant main effect of time ($P < 0.001$), with erythrocytes TBARS, protein carbonyls, and catalase generally increasing at 2, 3, and 4 d after exercise in both groups compared with baseline values.

There was no significant time × group interaction concerning GSH (Fig. 3A), GSSG (Fig. 3B), or GSH/GSSG

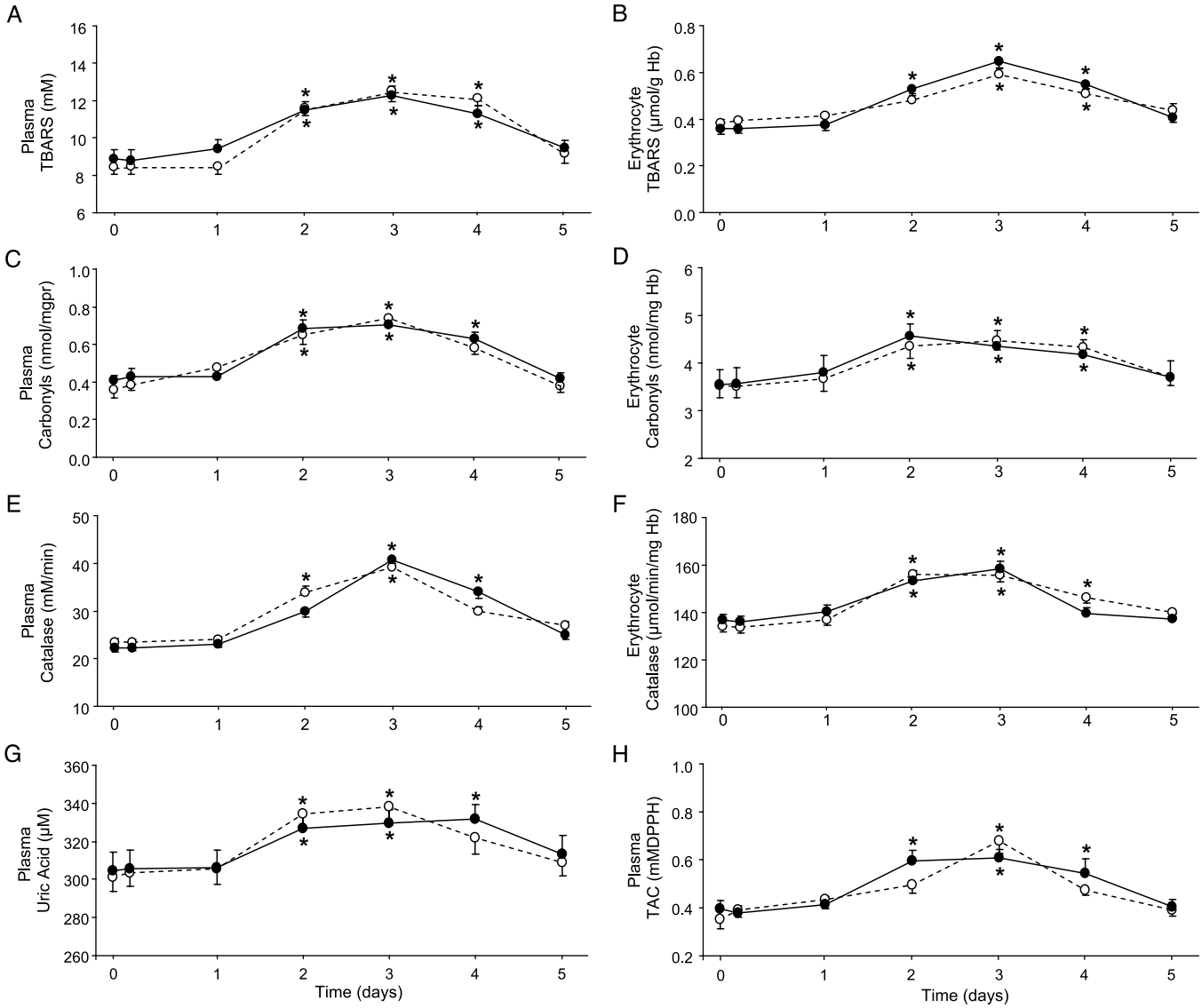

FIGURE 2—Plasma TBARS (A), erythrocyte TBARS (B), plasma protein carbonyls (C), erythrocyte protein carbonyls (D), plasma catalase (E), erythrocyte catalase (F), plasma uric acid (G), and plasma TAC (H) in the control (open circles) and G6PD-deficient individuals (closed circles) (mean ± SEM). *Significantly different from the preexercise value in the same group ($P < 0.05$). TBARS, thiobarbituric acid–reactive substances; TAC, total antioxidant capacity.

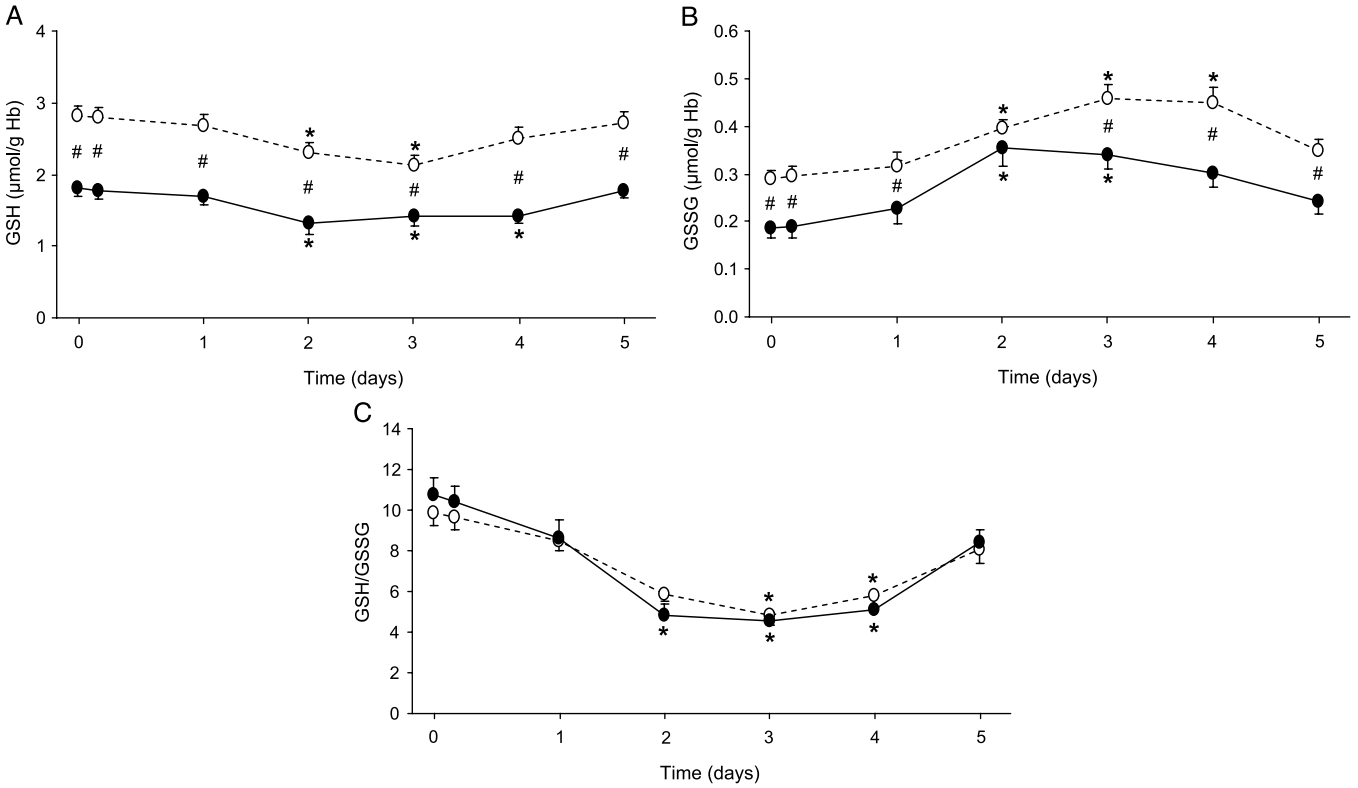

FIGURE 3—Erythrocyte GSH (A), GSSG (B), and GSH/GSSG (C) in the control (open circles) and G6PD-deficient individuals (closed circles) (mean ± SEM). *Significantly different from the preexercise value in the same group ($P < 0.05$). #Significantly different between the groups at the same time point ($P < 0.05$). GSH, reduced glutathione; GSSG, oxidized glutathione.

ratio (Fig. 3C). However, there was a significant main effect of group and time in GSH, GSSG, and GSH/GSSG ratio. Eccentric exercise decreased GSH, increased GSSG, and decreased GSH/GSSG ratio at several time points after exercise on both groups (main effect of time, $P < 0.05$). GSH level was 1.6 and GSSG level was 1.5 times higher at rest in the control group compared with the G6PD-deficient group (main effect of group, $P < 0.05$).

Hemolysis. There was no significant main effect of group or time × group interaction concerning plasma hemoglobin (Fig. 4A) and bilirubin (Fig. 4B). However, there was a significant main effect of time ($P < 0.001$), with plasma hemoglobin levels increasing immediately after and at 1, 2, 3, and 4 d and plasma bilirubin levels increasing at 1, 2, 3, and 4 d after exercise.

DISCUSSION

To our knowledge, this is the first investigation examining the effect of eccentric (i.e., muscle-damaging) exercise on muscle function, redox status, and hemolysis in G6PD-deficient individuals. Our hypotheses were that eccentric exercise would result in greater muscle damage, larger disturbances of systemic and erythrocyte redox status, and greater hemolysis in the G6PD-deficient individuals compared with the nondeficient counterparts. However, the present results failed to support these hypotheses; eccentric exercise similarly modified muscle function, blood redox status, and hemolysis in both groups.

Muscle function and damage. All muscle function and damage indices measured confirmed that eccentric exercise induced severe muscle damage, peaking at 2 and 3 d postexercise. These results are in accordance with similar investigations reporting intense and sustained muscle damage after eccentric exercise (19,28,32). Both the time course and the magnitude of the changes of the selected muscle function indices were similar between the two groups. This is regardless of the evidence supporting that increased levels of GSH enhance muscle performance (20,22,23,34,37). In fact, the calculated residual activity of G6PD (on the basis of a regression equation produced by Ninfali et al. (29) using erythrocyte G6PD activity) in skeletal muscle of our G6PD-deficient subjects was 10% of normal. The reasons G6PD-deficient individuals exhibited comparable muscle damage responses to nondeficient individuals are difficult to be predicted. The biochemical mechanism of NADPH (the cofactor responsible for the reduction of GSSG back to GSH) production in skeletal muscle is much more complicated than that of erythrocytes. In erythrocytes, NADPH is exclusively generated by the pentose phosphate pathway (6). On the contrary, in skeletal muscle and other tissues, $NADP^+$ (the oxidized form of NADPH) can also be generated by the action of NAD^+

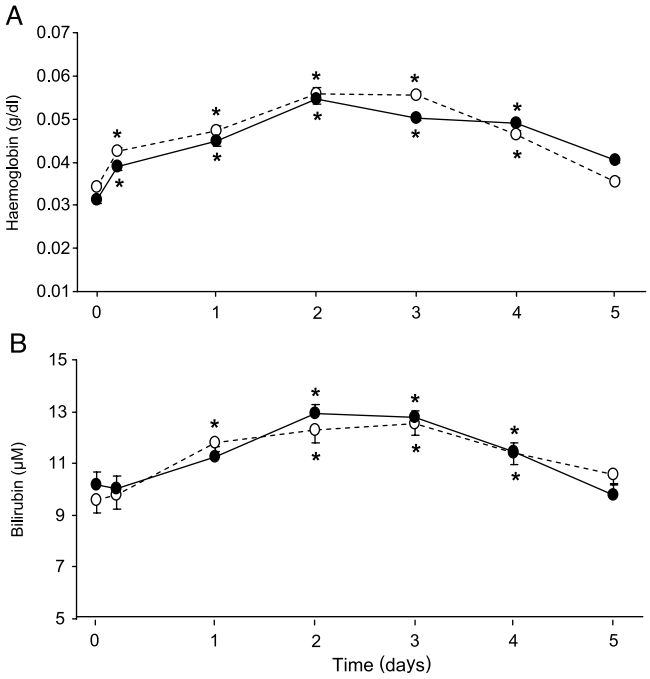

FIGURE 4—Plasma hemoglobin (A) and plasma bilirubin (B) in the control (open circles) and G6PD-deficient individuals (closed circles) (mean ± SEM). *Significantly different from the preexercise value in the same group ($P < 0.05$).

kinase and then reduced to NADPH by the action of malic enzyme (36) and/or $NADP^+$-dependent isocitrate dehydrogenase (1). Therefore, it is possible that NADPH production through malic enzyme and/or $NADP^+$-dependent isocitrate dehydrogenase is up-regulated in the skeletal muscle of G6PD-deficient individuals. In addition, it is likely that the presumed lower levels of GSH in skeletal muscle of G6PD-deficient individuals were merely sufficient to maintain thiol groups of myofibrillar proteins in a reduced state (11) and, ultimately, not affect muscle performance.

Blood redox status. In the present study, because of the complexity of redox homeostasis, a battery of redox status indices were measured to monitor changes in redox status as reliably as possible. In addition, different blood compartments were examined (i.e., plasma and erythrocytes). Plasma interacts with all organs and tissues and, consequently, with many possible sources of reactive species. Therefore, plasma is considered to reflect the redox status of whole body. In contrast, erythrocytes are less permeable, and their environment is more controlled than that of plasma and considered to reflect the redox status of a single type of cell.

We found lower resting levels of GSH in the G6PD-deficient group compared with the control group. This finding is in agreement with our previous work (18,27) and work of others (16,21). Because of its high concentration, GSH is considered the major antioxidant in erythrocytes (24). Notwithstanding, the levels of lipid and protein oxidation in erythrocytes were similar between the G6PD-deficient and the nondeficient individuals. This indicates that the lower by 34% levels of resting GSH in G6PD-deficient individuals are adequate to counteract the increased production of oxidants that appeared after eccentric exercise. This is not surprising considering the high concentration of GSH in erythrocytes and the fact that only few micromoles (≈0.3 μmol·g Hb^{-1} GSH) of GSH oxidized after eccentric exercise (even at day 2, erythrocytes of G6PD-deficient individuals contained ≈1.4 μmol·g Hb^{-1}). Therefore, in theory, the erythrocytes of G6PD-deficient individuals should have plenty of GSH to sustain a reduced environment. This is also underlined by the comparable relative decrease of GSH in the G6PD-deficient individuals (27%) and nondeficient individuals (24%), indicating that GSH regeneration rate is similar between the two groups. It is noteworthy that erythrocytes are characterized by the presence of hemoglobin at high concentration (8–10 mM), a value considerably larger than that of GSH (≈0.5 mM). Therefore, sulfhydryl groups of hemoglobin can have a significant role in the detoxification of oxidants by competing with GSH itself (12). As a final point, it is also possible that erythrocytes of G6PD-deficient individuals have developed alternative protective mechanisms from oxidation (other antioxidant molecules in the erythrocytes of G6PD-deficient individuals, such as vitamins C and E, may be at higher levels that those of nondeficient individuals).

Likewise, the degree of lipid and protein oxidation as well as the levels of antioxidant molecules in plasma was strikingly similar between the G6PD-deficient and the nondeficient individuals. Considering that plasma measurements reflect the overall redox status (including that of skeletal muscle), it is evident that no large tissue of G6PD-deficient individuals exhibited major perturbations in its redox status. This finding agrees with our previous findings (18,27), and those of others (3), where they did not show differences in plasma redox status between G6PD-deficient and nondeficient individuals. On the contrary, Jain and Palmer (17) reported higher levels of lipid peroxidation in both erythrocytes and plasma of G6PD-deficient individuals. We have no explanation for this discrepancy, but the different habitual diet of the participants (African Americans vs Mediterraneans) may partly explain this disagreement. A possible explanation for the similar plasma redox status between the two groups is that the G6PD-deficient individuals might have developed alternative protective mechanisms from oxidation offsetting their lower GSH level. The redox status indices determined in erythrocytes and plasma is noteworthy in the present study. Thus, although the level of antioxidant defenses measured in blood was similar between the two groups, in theory, the levels of these antioxidants in muscle might have been higher in G6PD-deficient individuals. Alternatively, the composition of muscle (and of any other cell affecting plasma and erythrocyte redox status) membranes may be different between the groups (e.g., fatty acid composition more resistant to peroxidation in G6PD-deficient compared with nondeficient individuals).

Eccentric exercise activated the antioxidant defenses of the body. Namely, catalase activity increased the day after eccentric exercise on both plasma and erythrocyte. TAC followed the same time course as catalase, peaking between 2 and 4 d, and uric acid resembled the changes of TAC supporting the well-described positive relationship between uric acid and TAC (38). This finding agrees with those of other studies where eccentric exercise induced prolonged increases in the levels of several antioxidants (26,28,31).

Hemolysis. The level of hemolysis was similar between the G6PD-deficient and the nondeficient individuals either at rest or after exercise. This fact denotes that erythrocytes of G6PD-deficient individuals are equally resistant to the hemolytic stimulus of eccentric exercise as well as corroborate the muscle damage and blood redox status data.

Increased short-lived hemolysis after non–muscle-damaging exercise is a well-described phenomenon in the relevant literature (33). Despite a controversy, the most probable reason for the aerobic exercise-induced hemolysis is the destruction of erythrocytes, resulting from the impact forces encountered during exercise (33). Here, to the best of our knowledge, we describe for the first time marked and long-lived increases in hemolysis after eccentric exercise. Increased levels of plasma hemoglobin and bilirubin were found up to 4 d after exercise, peaking between 2 and 3 d, and not immediately after it. Undoubtedly, the prolonged increase in hemolysis after eccentric exercise cannot be attributed to mechanical stress, because the subjects remained at rest when the hemolysis peaked. It is noteworthy that hemolysis indices followed the same time course of those of muscle damage and redox status indices. This observation implies that the degree of muscle damage and oxidative stress after eccentric exercise determines to some extent the degree of hemolysis. Indeed, leukocytes are activated after eccentric exercise and release many free radicals (4,9). The oxidative stress that follows may lead to lipid peroxidation and concomitant membrane damage and erythrocyte death (7). This is facilitated by the high polyunsaturated fatty acid and iron content of erythrocytes (7). Summarizing, we believe that free radicals released mainly by leukocytes after eccentric exercise to repair damaged muscle tissue are partially responsible for the prolonged hemolysis observed the days after eccentric exercise.

Participation of G6PD-deficient individuals in physical activities. To the best of our knowledge, the only studies relevant to the effect of exercise on G6PD-deficient subjects (except those from our group) are three case studies (5,10,30). The case studies by Bresolin et al. (5) and Ninfali et al. (30) reported that one G6PD-deficient individual (in each study) was hospitalized for myalgia and myoglobinuria after intense exercise. Nevertheless, it is not clear whether these clinical symptoms appeared because of the G6PD deficiency or were just a result of a preceding severe exercise bout. Indeed, myalgia and myoglobinuria can also be found in non–G6PD-deficient individuals after intense, particularly eccentric, exercise (13,25). It is therefore probable, in our opinion, that the symptoms reported in these studies were due to a preceding exercise bout and were not caused by the G6PD deficiency itself. Contrary to both these reports, a recent case study (10) reported that an elite long-distance runner athlete showed no more severe signs of myalgia, myoglobinuria, or hemoglobinuria than other elite long-distance runners. In agreement with Demir et al. (10), our previous full-scale studies (18,27) performed under controlled environmental conditions showed that G6PD-deficient individuals are not more susceptible to oxidative damage and erythrocyte destruction than nondeficient individuals after high-intensity aerobic exercise. In accordance with the previous studies, the present study lends support to the idea that the muscle damage and redox status responses of G6PD-deficient are comparable to nondeficient individuals, even after intense eccentric exercise.

CONCLUSIONS

In the present study, for the first time to our knowledge, muscle function and redox status were measured to examine whether G6PD-deficient individuals are less capable to perform muscle work and/or are more vulnerable to systemic and erythrocyte damage than nondeficient individuals. Nevertheless, and despite our hypotheses, muscle function and redox status of G6PD-deficient individuals either at rest or after eccentric exercise were very similar to those of controls. The present work indicates that G6PD enzyme is not a critical component of the cellular antioxidant system in erythrocytes and is not required for maintaining contractile function of skeletal muscle in G6PD-deficient individuals. Furthermore, the hemolysis observed in the days after exercise and specifically in the days where muscle damage and redox status indices exhibited the greatest changes suggests that free radicals might be a major contributor for this implication. The present and our previous results (18,27) oppose the concerns (5,30) regarding the participation of these individuals in physical activities and imply that G6PD-deficient individuals may participate in various physical activities, even high-intensity muscle-damaging activities, without a negative impact on muscle function and blood redox status.

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