Whey Protein Inhibits Iron Overload-Induced Oxidative Stress in Rats

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Summary In this study, we evaluated the effects of whey protein on oxidative stress in rats that were subjected to oxidative stress induced by iron overload. Thirty male rats were assigned to 3 groups: the control group (regular [50 mg/kg diet] dose of iron + 20% casein), iron overload group (high [2,000 mg/kg] dose of iron + 20% casein, IO), and whey protein group (high dose of iron + 10% casein + 10% whey protein, IO + whey). After 6 wk, the IO group showed a reduction in the plasma total radical trapping antioxidant parameter and the activity of erythrocyte superoxide dismutase and an increase in lipid peroxidation (determined from the proportion of conjugated dienes). However, whey protein ameliorated the oxidative changes induced by iron overload. The concentration of erythrocyte glutathione was significantly higher in the IO + whey group than in the IO group. In addition, whey protein supplementation fully inhibited iron overload-induced DNA damage in leukocytes and colonocytes. A highly significant positive correlation was observed between plasma iron levels and DNA damage in leukocytes and colonocytes. These results show the antioxidative and antigenotoxic effects of whey protein in an in vivo model of iron overload-induced oxidative stress.

Key Words whey protein, iron overload, oxidative stress, antioxidant, DNA damage

Whey is a by-product of casein precipitation, an important step in the manufacture of cheese. It has become a valuable food ingredient because of its excellent nutritional value and functional properties. It constitutes approximately 85–90% of the volume of milk that is used to manufacture ripened cheese, and it retains approximately 55% of the milk nutrients (1, 2). The major constituents of bovine whey protein (WP) are β-lactoglobulin (55–60%) and α-lactalbumin (15–20%). The minor constituents include bovine serum albumin, lactoferrin, immunoglobulins, glycomacropeptides, phospholipoproteins, bioactive factors, and enzymes (3–5).

Several functional and biological activities of WPs have been reported, such as opioid inhibition, inhibition of angiotensin I-converting enzyme, antihypertensive activity, immunomodulatory activity, bacteriostatic activity, and downregulation of fatty acid synthesis in the liver (1, 6–9). WP possesses antioxidant activity, which has been recognized as the factor responsible for the chelation of transition metals by serum albumin and lactoferrin, an iron-binding glycoprotein, as well as for the free radical scavenging activity shown by amino acids such as tyrosine and cysteine (10–13). The in vivo antioxidant effects of WP have been demonstrated in the heart tissue of iron-treated mice and in the plasma and liver tissue of vitamin E-deficient rats (14, 15).

Many pro-oxidant drugs and other chemicals have been implicated in the oxidative stress and cell injury that result from the intracellular production of harmful oxygen radicals (16). Transition metals, such as iron and copper, with their multiple oxidation states are vital for the cascades of electron transfer reactions. These metals participate in cellular processes such as oxygen transport, photosynthesis, nitrogen fixation, and respiration in most organisms (17–19). Iron is the most abundant transition metal in the human body; is primarily present in protein-bound forms, such as in heme and non-heme proteins; and plays a crucial role in electron transfer and oxygen utilization reactions (20). Iron exposure is directly associated with the pathogenesis of many disorders, such as atherosclerosis, cancer, and inflammation, possibly via the production of reactive oxygen species (ROS) (19).

The results of recent in vitro and in vivo (animal) studies have shown that certain dietary antioxidants protect against iron-induced oxidative stress (21, 22). However, the antioxidant effect of WP on iron-induced oxidative stress has not been studied thoroughly. In this study, we investigated the effect of WPs on oxidative stress in rats subjected to iron overload-induced oxidative stress.

MATERIALS AND METHODS

Preparation of WP. WP (40% protein) was prepared from fresh mozzarella cheese whey in the laboratory by ultrafiltration.

Animal and diets. Eight-week-old male Sprague Dawley rats (n = 30) were purchased from Samtako Inc. (Osan, Korea). They were housed and cared for in accor-

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Table 1. Composition of the experimental diet.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IO</th>
<th>IO+WP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>WP</td>
<td>—</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>55.924</td>
<td>54.959</td>
<td>54.959</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mixture$^1$</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Iron (FeSO₄·7H₂O)</td>
<td>0.025</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

$^1$ Control: normal control group, IO: iron overload group, IO+WP: iron overload+whey protein.

$^2$ The AIN 76 vitamin mixture contained (in g/kg of mixture) the following: thiamine HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; niacin, 3.0; d-calcium pantothenate, 1.6; folic acid, 0.2; d-biotin, 0.02; cyanocobalamin (vitamin B₁₂), 0.001; dry vitamin A palmitate (500,000 U/d), 0.8; dry vitamin E acetate (500 U/d), 10; vitamin D₃; trituration (400,000 U/g), 0.25; menadione sodium bisulfite complex, 0.15; finely powdered sucrose, 981.08.

$^3$ The AIN 76 mineral mixture contained (in g/kg of mixture) the following: calcium phosphate, dibasic, 500; sodium chloride, 74; potassium citrate, monohydrate, 220; potassium sulfate, 52; magnesium oxide, 24; manganous carbonate (43–48% Mn), 3.5; ferric citrate (16–17% Fe), 6; zinc carbonate (70% ZnO), 1.6; cupric carbonate (53–55% Cu), 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium potassium sulfate, 0.55; finely powdered sucrose, 118.03.

dance with the Guide for the Care and Use of Laboratory Animals (23). All experimental protocols for animal care and use were approved by the Institutional Animal Care and Use Committee at Kyungnam University, Changwon. The rats were acclimatized to the animal facility room in Kyungnam University for 1 wk. They were housed individually (20–22°C; 12:12-h light-dark cycle) with free access to a commercially prepared pelleted diet and water. The rats were then randomly divided into 3 groups of 10 animals each and fed either a standard diet (control group), a standard diet supplemented with 0.2% ferrous iron (iron overload group, IO), or a standard diet supplemented with 0.2% ferrous iron+10% whey protein (IO+whey group) for 6 wk (Table 1). We fed 0.25 g Fe (FeSO₄·7H₂O)/kg dry matter (DM) to the control group and 9.9 g Fe/kg DM to the iron-overload group, according to the method described by Lafay et al. (21). The animals were monitored daily for general health, and body weights were recorded every week for the duration of the study. At the end of the experimental period, the rats were anesthetized with ethyl ether. The colon was removed for the comet assay, and blood was collected from the abdominal artery in a heparinized sterile tube. Whole blood was freshly prepared for the comet assay. Plasma was obtained from the blood samples by centrifugation (1,500 rpm for 30 min) and stored at −80°C until required for further analysis. Erythrocytes were washed 3 times with isosmotic phosphate-buffered saline (PBS, pH 7.4) and resuspended to the original volume. The erythrocyte suspensions were frozen at −80°C until they were required for the final analysis. The livers were removed from the rats and washed with ice-cold saline, at which time they were stored at −80°C before analysis.

**Total plasma iron.** The total plasma iron concentration was determined using an assay kit (QuantiChrom™ Iron Assay Kit, BioAssay Systems, Hayward, CA) based on the ferrozine spectrophotometric technique.

**Plasma total radical trapping antioxidant potential.** The plasma total radical trapping antioxidant potential (TRAP) was measured using a modification of the photometric method developed by Rice-Evans and Miller (24). The method used for measuring antioxidant activity is predicated on the antioxidant-induced inhibition of the absorbance of the radical cation of 2,2'-azino-bis (3-ethylbenzothiazoline 6-sulfonate) (ABTS$^+$). The ABTS$^+$ radical cation is formed by interaction between ABTS$^+$ (150 μM) and the ferryl myoglobin radical species, which, in turn, is generated by the activation of metmyoglobin (2.5 μM) by H₂O₂ (75 μM). Ten microliters of sample/buffer/Trolox-standard was added to tubes containing 400 μL of PBS, 20 μL of metmyoglobin, and 400 μL of ABTS and mixed by vortexing. The reaction was initiated by the addition of the 170 μL of H₂O₂. After 6 min of incubation, the absorbance was measured at 734 nm using a spectrophotometer. Values have been expressed in terms of Trolox equivalent antioxidant capacity (TEAC) and defined in terms of the molar concentration of the Trolox antioxidant capacity of a calibration curve.

**Baseline levels of conjugated dienes in low-density lipoprotein.** Baseline low-density lipoprotein (LDL)-conjugated diene levels were determined according to the methods outlined by Ahotupa et al., with slight modifications (25). Plasma (100 μL) was added to 700 μL of heparin citrate buffer (0.064 m trisodium citrate; 50,000 IU/L heparin; pH 5.05), and the suspension was incubated for 10 min at room temperature. The insoluble lipoproteins were then sedimented by centrifugation at 2,500 rpm for 10 min. The pellet was resuspended in 100 μL of 0.1 m Na-phosphate buffer containing 0.9% NaCl (pH 7.4). Lipids were extracted from 100 μL of the LDL suspension with chloroform-methanol (2:1), dried under a nitrogen atmosphere, dissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm. Oxidation during sample preparation was prevented by the addition of ethylenediaminetetraacetic acid (EDTA).

**Plasma lipid-soluble vitamins.** Plasma concentrations of retinol, carotenoids, α-tocopherol, and coenzyme Q₁₀ were determined simultaneously by reversed-phase high pressure liquid chromatography (RP-HPLC) according to the method reported by Jakob and Elmadfa (26). Briefly, plasma proteins were precipitated with ethanol,
and lipids were extracted with n-hexane. After evaporation, the dry residue was dissolved with 150 μL of methanol-dichloromethane (85:15, v/v) and mixed. One hundred microliters of this solution was injected into a guard-column (Merck LiChrospher 100 RP18 [10 μm], 250×4 mm). Samples were run at a flow rate of 1.0 mL/min on a Summit™ HPLC system ( Dionex, Sunnyvale, CA). Absorption was measured at 325 nm for retinol, 295 nm for α-tocopherol, 450 nm for carotenoids, and 270 nm for coenzyme Q10. Concentrations were calculated from the areas under the curve by using an external calibration curve.

**Erythrocyte antioxidant enzyme activities.** Glutathione-peroxidase (GSH-Px) activity was determined according to the method described by Beutler (27). Ten microliters of erythrocytic hemolysate was added to 100 μL of 1 m Tris-HCl−5 mM EDTA buffer (pH 8.0), 20 μL of 0.1 m glutathione, 100 μL of 10 U/mL glutathione reductase, and 100 μL of 2 mM NADPH. H2O2 was added to obtain a final volume of 1 mL. After 10 min of incubation at 37°C, the reaction was initiated by the addition of 10 μL of tert-butyl hydroperoxide, and the absorbance was measured at 340 nm. The reaction was run for 90 s, and the oxidation of NADPH was monitored by the change in the absorption at 340 nm/min values.

Catalase (CAT) activity was measured according to the method developed by Aebi (28). One hundred microliters of erythrocytic hemolysate was dissolved in 50 mL of 50 mM phosphate buffer (pH 7.0), and 2 mL of the mixture was added to a cuvette. The reaction was initiated by the addition of 1 mL of 0.1 M pyrogallol. The H2O2 decomposition rate was measured at 240 nm for 30 s by using a spectrophotometer.

The activity of superoxide dismutase (SOD) was assayed in the erythrocyte suspension by using the procedure reported by Marklund and Marklund (29). Briefly, 3.5 mL of water, 1 mL of ethanol, and 0.6 mL of chloroform were added to 500 μL of hemolysate. After the mixture was centrifuged at 3,000 rpm for 2 min, various dilutions were prepared from the supernatant. After the diluted solutions were incubated at 37°C for 10 min, 20 μL of pyrogallol (10 mM) was added to each dilution. The reaction was monitored spectrophotometrically at 320 nm for 2 min. A unit of enzyme was defined as the amount that inhibited the autoxidation of pyrogallol by 50%.

The activity of glutathione-S-transferase (GST) was assayed by the method reported by Habig et al. (30), using 0.12 mM 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. Fifty microliters of hemolysate was added to 2,935 μL of 0.1 M phosphate buffer containing 0.1 M NaH2PO4, 0.1 M K2HPO4, and 30 μL of 0.1 M glutathione (GSH). CDNB (25 μL) was added to start the reaction. The changes in optical density were recorded at 340 nm for 3 min. The enzyme activity was calculated using an extinction coefficient of 9.66 mM cm−1. The hemoglobin contents of the erythrocytic hemolysates were determined using an assay kit (BCS, Anyang, Korea). The activities of SOD, CAT, GSH-Px, and GST were calculated in terms of U/g Hb.

The GSH concentrations in erythrocyte suspensions were measured using a GSH assay kit (Calbiochem, San Diego, CA).

**DNA damage determination by the alkaline comet assay.** Colon cells were isolated from dissected colon tissue for cytotoxicity and genotoxicity analyses. The alkaline comet assay was conducted according to the protocols established by Singh et al. (31), with little modification. Frosted slides (Fisher Scientific) were prepared with a basal layer of 0.5% normal melting agarose as follows: 5 μL of whole blood or a colon cell suspension (2×109 colon cells/L) was mixed with 75 μL of 0.7% low melting agarose (LMA) and then added to the slides. The slides were covered with cover slips and kept in a refrigerator for 10 min. The cover slips were then removed, and a top layer of 75 μL of 0.7% LMA was added before placing the slides (with cover slips) in a refrigerator again for 10 min. After removal of the cover slips, the slides were immersed in a jar that contained cold lysing solution (pH 10.0) consisting of 2.5 mM NaCl, 100 mM EDTA, 10 mM Tris, and 1% sodium laurylsarcosine. Subsequently, 1% Triton X-100 and 10% DMSO were freshly added to the solution, which was then stored in a refrigerator for 1 h. After lysis, the slides were placed in a horizontal electrophoresis tank (Threeshine Co. Ltd., Daejeon, Korea). The slides were covered with fresh alkaline buffer (300 mM NaOH, 10 mM Na2EDTA, pH 13.0) and maintained at 4°C for 40 min. DNA was electrophoresed by applying an electric current of 25 V/300–3 mA for 20 min at 4°C. The slides were washed 3 times with neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4°C and then treated with ethanol for another 5 min. All the steps following the lysis treatment were undertaken in darkness in order to prevent additional DNA damage. Fifty microliters of ethidium bromide (20 μg/mL) was added to each slide, and the slides were examined using a fluorescence microscope (LEICA DMLB, Wetzler, Germany). Images of 100 randomly selected cells (50 cells from each of 2 replicate slides) were analyzed for each subject. Measurements were made with image analysis software (Komet 5.0, Kinetic Imaging, Liverpool, UK) to determine the percentage of DNA in the tail (tail intensity).

**Statistical analysis.** Data were analyzed using the SPSS package for Windows (Version 12, SPSS Inc., Chicago, IL). Values are expressed in terms of mean±standard error (SE). The data were evaluated by one-way ANOVA, and the difference between the means was assessed using Tukey’s test when the F value was significant. The differences were considered significant at p<0.05. Pearson’s correlation was used to evaluate the associations between parameters.

**RESULTS**

**Food intake, weight gain, and organ weight**

During the experiment, no signs of treatment-associated adverse effects were observed in the clinical appearance of the animals. No differences in weight gain, food intake, or food efficacy ratio were observed among the 3 groups (Table 2). The significant increase in relative
Antioxidant Effect of Whey Protein

Heart weight due to iron overload was corrected by whey protein supplementation.

Plasma total iron

The plasma iron concentration in the IO group was significantly higher than that in the control group (316.9 ± 20.4 μg/dL vs. 265.0 ± 20.5 μg/dL; p = 0.038) (Fig. 1). Supplementing the diet with 10% whey protein lowered the plasma iron concentration by 17% in the IO+Wp group (263.4 ± 18.8 μg/dL; p = 0.028).

Plasma antioxidant potential and lipid peroxidation

Iron overload reduced the plasma TRAP, an indicator of total antioxidant defense, and increased the proportion of plasma-conjugated diene, a marker of lipid peroxidation (Fig. 2). Whey protein supplementation led to a 3.7% increase in the TRAP values (p < 0.01) and a significant 40.7% reduction in the proportion of plasma-conjugated diene (p < 0.05) in the IO+Wp group, when compared with the corresponding values in the IO group.

Other antioxidant-related parameters in the plasma and erythrocytes

Table 3 shows the effect of whey protein supplementation on the plasma concentrations of antioxidant vitamins and the activities of erythrocyte antioxidant enzymes in rats fed an iron-rich diet. The erythrocyte SOD activity in the IO group was significantly lower (44.8% lower) than the activity in the control group. However, whey protein supplementation significantly increased the erythrocyte SOD activity. The IO+whey group showed a significant increase in the concentration of erythrocyte GSH compared with that in the IO group. No significant changes were observed in the plasma concentrations of antioxidant vitamins or the activities of other erythrocyte enzymes.

Table 2. Effects of whey protein on body weight gain, food intake, and FER in rats fed an iron overload diet.

<table>
<thead>
<tr>
<th></th>
<th>Control1</th>
<th>IO</th>
<th>IO+WP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g/d)</td>
<td>3.7±0.1ns3</td>
<td>3.5±0.2</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>18.5±0.4sm</td>
<td>18.6±0.4</td>
<td>18.8±0.3</td>
</tr>
<tr>
<td>FER</td>
<td>19.4±0.5sm</td>
<td>18.5±1.2</td>
<td>17.7±0.8</td>
</tr>
<tr>
<td>Organ weight (g/100 g BW)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>4.18±0.11ns</td>
<td>4.09±0.08</td>
<td>3.96±0.11</td>
</tr>
<tr>
<td>Heart</td>
<td>0.54±0.02sm</td>
<td>0.59±0.01b</td>
<td>0.53±0.02a</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.96±0.02ns</td>
<td>0.97±0.02</td>
<td>0.94±0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.30±0.01ns</td>
<td>0.33±0.02</td>
<td>0.30±0.01</td>
</tr>
</tbody>
</table>

Values are the mean±SE for 10 animals in each group.
1 Control: normal control group, IO: iron overload group, IO+WP: iron overload+whey protein.
2 FER, food efficiency ratio.
3 ns, not significant.
4 The values in the same row that do not share a common superscript are significantly different at the p < 0.05 level.

Fig. 1. Effects of whey protein on the plasma Fe concentration in rats fed an iron overload diet. Control: normal control group, IO: iron overload group, IO+WP: iron overload+whey protein. Each bar represents the mean±SE value for 10 animals in each group. Bars with different superscript letters are significantly different at p < 0.05 by Tukey’s test.

Fig. 2. Effects of whey protein on plasma total antioxidant potential (TRAP) and lipid peroxidation (conjugated dienes, CD) in rats fed an iron overload diet. Control: normal control group, IO: iron overload group, IO+WP: iron overload+whey protein. Each bar represents the mean±SE for 10 animals in each group. Bars with different superscript letters are significantly different at p < 0.05 by Tukey’s test.
Leukocyte and colonocyte DNA damage

Figure 3 shows the effects of whey protein supplementation on iron overload-induced DNA damage in the peripheral blood cells and colon cells of rats. Iron overload induced a significant increase in the proportion of strand breaks in leukocyte DNA (IO vs. control: 3.4±0.2 vs. 17.0±0.7%; p<0.001) and colonocyte DNA (IO vs. control: 11.2±1.9 vs. 53.7±1.6%; p<0.001). In rats fed a diet supplemented with 10% whey protein for 5 wk, the strong genotoxic effect of iron overload was reduced by 62% in leukocytes and by 87% in colonocytes.

Plasma iron concentrations showed a highly significant positive correlation with DNA damage in leukocytes (r=0.456, p=0.013) and in colonocytes (r=0.382, p=0.041) (Fig. 4).

**DISCUSSION**

The aim of this study was to determine whether increased oxidative stress due to iron overload could
be ameliorated by dietary supplementation with whey protein.

We found that dietary supplementation with whey protein for 6 wk was effective in ameliorating the oxidative stress induced by dietary iron overload. Whey protein supplementation significantly increased the plasma TRAP values and erythrocyte SOD activity and decreased the levels of plasma lipid peroxidation.

Free iron available from ferrous sulfate supplementation mainly exists as insoluble ferric Fe(III) at physiological pH and in the presence of oxygen (32). Since iron transport systems are specific for ferrous Fe(II), ferric iron is reduced by duodenal ferric reductase (33), thus allowing cells to take up iron. Highly unstable ferrous iron (Fe^{2+}) catalyzes the conversion of hydrogen peroxide to ROS, such as hydroxyl (•OH) and superoxide (O$_2^−$) radicals, through Fenton-type or iron-catalyzed Haber-Weiss biochemical reactions. These ROS can damage important biomolecules, namely lipids, proteins, and DNA (34–36). The results of in vitro and in vivo studies have shown that iron overload can enhance oxidative stress and increase DNA strand breakage and the oxidation of DNA bases (37, 38). Our results showed that consumption of a diet supplemented with 0.2% ferrous iron for 5 wk can significantly increase plasma iron concentrations and consequently oxidative stress, which was evident from the decrease in the plasma TRAP values and erythrocyte SOD activity and the increase in the levels of plasma lipid peroxidation.

The oxidative stress induced by iron overload caused DNA damage in peripheral leukocytes and colonocytes; the damage observed was almost 5 times that in the control group. Moreover, a significantly positive correlation between the plasma iron concentration and the level of DNA damage was observed in leukocytes and colonocytes. Our findings are consistent with the findings of Rehman et al. (39), who reported that dietary supplementation with iron sulfate and vitamin C increased the level of oxidative damage in the white blood cells of healthy humans. Furthermore, DNA damage in the lymphocytes of patients with thalassemia, a genetic hematological disorder characterized by an increase in iron concentration that results from the impairment of hemoglobin synthesis, was 4 times higher than that observed in the lymphocytes of normal individuals (40). The presence of unabsorbed free iron moieties, which are produced as a result of dietary supplementation, has been reported to increase the rate of free radical formation in the colon of healthy volunteers to a level that can cause mucosal cell damage (32). SOD activity in erythrocytes, whose potent enzymatic and nonenzymatic antioxidant activities modify highly ROS into substantially less reactive intermediates (41), was decreased by iron overload.

Whey protein inhibited iron overload-induced damage to DNA in leukocytes and colonocytes. Bartfai et al. (14) reported an in vivo antioxidant effect of whey protein; they showed that mice receiving iron treatment (10 mg/dL of iron dextran, i.p.) supplemented with whey protein had significantly lower levels of cytotoxic aldehydes and higher levels of glutathione peroxidase (GSH-Px) and GSH in heart tissue than mice treated with iron alone. Zommarra et al. (15) showed that whey protein supplementation increases the concentrations of α-tocopherol and GSH in the plasma and liver, respectively, whereas it decreases lipid peroxidation in the liver of rats fed a diet low in vitamin E.

The mechanism underlying the antioxidant effects of whey protein on iron overload-induced oxidative stress may involve the iron-chelating properties of whey protein and lead to increased iron excretion in urine and feces. This assumption is supported by the significant reduction in plasma iron concentrations in the IO+WP group observed in the present study. Lactoferrin, a major component of whey protein, is believed to chelate trace metals, including iron (6, 42). As reported by Bihel and Birlouez-Aragon (43), the binding of iron to lactoferrin may decrease the rate at which hydrogen peroxide is converted to hydroxyl radical via the Fenton reaction, thereby inhibiting the oxidation of ascorbic acid and tryptophan.

Another possible mechanism for the antioxidant effect of whey protein involves its ability to scavenge free radicals. The results of in vitro studies showed that β-lactoglobulin and serum albumin, which are predominant proteins in whey, contain 1 free sulf hydryl group that scavenges free radicals, thereby inhibiting lipid peroxidation (6, 44).

The cysteine-rich proteins in whey, which aid in the synthesis of GSH, a potent intracellular antioxidant (45), could also contribute to the antioxidant effect of whey. The results of some studies have shown that iron overload induces GSH depletion in the serum, liver, and heart tissue (19, 40). Although a diet supplemented with 0.2% ferrous iron did not appear to affect the GSH levels in erythrocytes, supplementation of an iron-rich diet with whey protein enhanced GSH synthesis in erythrocytes by 31%. We believe that the increased GSH concentration helped enhance the cellular defense mechanisms against ROS formation that is induced by iron overload in the whey protein-supplemented group.

In conclusion, the findings of our study of an in vivo model of oxidative stress induced by iron overload clearly indicate that whey protein protects against iron-mediated ROS formation and thereby protects against DNA damage in white blood cells and in the colon. Further analyses of the antioxidant status in other organs (e.g., liver and heart) are required to understand the exact mechanism underlying the protective effects of whey protein.

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REFERENCES
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