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Original Article

The Effects of High-Dose Whey Protein Concentrate Intake on Hepatorenal and Intestinal Tissues

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Abstract

Introduction: This study aims to compare the antioxidant effects of high doses of whey protein (WP) concentrate intake on liver, kidney, and intestinal tissues.

Methods: 18 rats were divided into three groups control (n=6), control+ 8 g/kg WP (n=6), and control + 2 g/kg WP (n=6). 8 g/kg WP group was fed with whey protein added rat chow. 2 g/kg WP group, addition to their standard ad libitum feed, received the whey protein concentrate by oral gavage. On day 10, liver, kidney and intestinal tissues were removed. Lipid peroxidation, glutathione (GSH) levels, superoxide dismutase (SOD) and glutathione-S- transferase (GST) activities were determined in liver, kidney and intestine tissues.

Results: GSH, SOD, and GST activities increased in whey protein-administered groups. Liver glutathione level was higher in the 2 g/kg WP compared to the 8 g/kg WP group. There were no significant differences in intestinal glutathione levels between the groups. Kidney GST activity decreased in the kidney and intestine tissues of the 8 g/kg WP group compared to the 2 g/kg WP group. SOD activity was higher in all tissues in the 2 g/kg WP group compared to the other groups.

Conclusion: As a result, both whey protein treatments showed different antioxidant effects in the tissues examined. High-dose whey protein application showed lower antioxidant capacity compared to the optimal whey protein dose.

Keywords: High dose whey protein, antioxidant activity, liver, kidney, intestine

1. Introduction

Whey proteins are a group of highly beneficial proteins derived from the liquid component of milk that separates during cheese production. These proteins hold considerable importance due to their amino acid composition, which gives them

the highest biological value among all protein sources (1). Whey protein-rich diets offer protection from some human diseases; however, there is no study on whether their effect changes when taken with food. Whey proteins are not only an excellent source of high-quality nutrients but are also quickly absorbed and utilized by the human body. They provide numerous health benefits, such as promoting muscle growth, supporting weight management, reducing inflammation, and improving digestion (2).

Whey proteins have a high level of radical scavenging action, which can be attributed to the numerous peptides included in them. Previous research has shown that whey proteins can inhibit oxidative damage in the iron-catalyzed liposome oxidation system (3). One of the advantages of using whey protein as a source of antioxidants is its high bioavailability. Whey protein is easily absorbed by the body and can be quickly transported to cells, where it can exert its antioxidant effects (4). They have rich cysteine amino acid content, which has a thiol group that interacts with glycine and glutamate to make GSH, the principal antioxidant of the cells. GSH detoxifies toxic substances such as toxic metals, lipid peroxides, and prostaglandins. Cysteine controls GSH concentrations; hence, supplementing the diet with whey protein high in cysteine can increase GSH production (5). Whey proteins are digested more slowly than casein and remain in the intestines for a longer period compared to casein. As a result, whey protein is more beneficial to the body and has the potential to promote human health, and it may be employed as a treatment method for oxidative stress-related disorders (6).

A high-protein diet can help to lose weight and improve insulin sensitivity, but it's unclear if the kind of protein to be taken affects these benefits. The high dose whey proteins usage may have negative effects on health. The negative consequences include a rise in acne, microbiota malfunction, and changes to the kidneys' normal metabolic process (7). Therefore, it is crucial to reasearch how different doses of whey proteins affect particular tissues. In this study, the antioxidant effects of high-dose whey protein application on liver, kidney and intestinal tissues were investigated.

2. Methods

2.1. Preparation of Whey Protein Added Rat Chow

Rat chows were pulverized using a laboratory grinder. 100 g of powdered chow was mixed with 200 mL of whey protein concentrate (Tazelen, Kaanlar Food Industry and Trade, Turkey). The mixture, which was brought to the consistency of dough, was given its first shape. The prepared chow was dried using a lyophilizer at -50^{o}C. 158 g of chow containing 20 g of protein was obtained. This dried chow was given to the rats. The feed consumption of the rats was monitored.

2.2. Animals and Experimental Design

Sprague Dawley rats (male, 200–300 g, n=18) were kept in standard animal laboratory conditions with light and dark (12h/12h) cycles. Control group rats (n=6) were fed with standard rat chow and tapped water ad libitum. Control+whey with chow (8 g/kg WP) group rats (n=6) were fed with prepared whey protein added chow and tap water ad libitum. 2 g/kg WP group rats (n=6) were fed with rat chow tap water ad libitum and also was given whey protein concentrate (2 g/kg).

2.3. Determination of the Lipid Peroxidation and Glutathione (GSH) Levels

Hepatorenal and intestinal malondialdehyde (MDA) levels were determined by the method of Ledwozyw for the detection of lipid peroxidation (8). In summary, the compound that is produced by boiling tissue homogenate with thiobarbituric

acid is extracted using n-butanol. The variance in optical density at 532 nm is calculated using the MDA level which serves as an indicator of lipid peroxidation. The results were presented as nmol MDA/g tissue. The GSH level in these tissues was measured by the method of Beutler (9). The process involves reducing Ellman's reagent with SH groups to generate 5,5′-dithiobis (2-nitrobenzoic acid), which has a yellow color and is measured spectrophotometrically at 412 nm.

2.4. Determination of the Superoxide dismutase (SOD) and Glutathione-S-Transferase (GST) Activities

Superoxide dismutase (SOD) activity was determined by the capacity of riboflavin-sensitized o- dianisidine to increase the rate of photooxidation (10). 2.8 mL 50 mM potassium phosphate (pH = 7.8) with 0.1 mM EDTA, 0.2 mM riboflavin in 10 mM potassium phosphate (pH 7.5), 0.1 mL 6 mM *o*-dianisidin, and tissue extract were mixed. Cuvettes with all their components were illuminated with 20-W Slylvania Grow Lux fluorescent tubes maintaining the temperature of 37°C. Absorbance was measured at 460 nm and the result expressed in U SOD per g tisssue. Glutathione-S-transferase (GST) activity was determined by measuring the absorbance at 340 nm of the product produced by the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) (11). Glutathione and 1-chloro2,4-dinitro-benzene conjugation product was measured at 340 nm. The GST activity was calculated using the extinction coefficient (9.6 mM-1 cm-1) obtained for the compound produced by the conjugation of glutathione and 1-chloro-2,4-dinitrobenzene. The results were expressed in U/g tissue.

2.5. Statistical Analysis

Analysis of variance (ANOVA) and Tukey, multiple comparison tests, were carried out using GraphPad Prism 9.0 (California, USA). $p < 0.05$ was considered significant.

3. Results

MDA levels in all tissues did not change significantly with whey protein applications in all groups (Fig. 1). Glutathione levels increased in liver and kidney tissues but did not change in the intestine tissue both in the 8 g/kg WP and 2 g/kg WP groups compared to the control group. Liver GSH level was also significantly higher than the 8 g/kg WP group in the 2 g/kg WP group (Fig. 2). Liver and intestine SOD activities increased both in the 8 g/kg WP and 2 g/kg WP groups compared to the control group. In the 2 g/kg WP group, intestinal SOD activity was also significantly higher than the 8 g/kg WP group (Fig. 3). GST activity did not significantly change in liver tissue in all groups. Kidney and intestine GST activities decreased in the 8 g/kg WP groups compared to the control group. In the 2 g/kg WP group, intestinal GST activity was also significantly higher than in the 8 g/kg WP group (Fig. 4).

4. Discussion

This study aims to investigate the antioxidant effects of high-dose whey protein concentrate on the liver, kidney, and intestinal tissues of healthy rats. Whey proteins have antioxidant characteristics due to their high concentrations of bioactive peptides and amino acids, including cysteine, methionine, and glutamine. These components are thought to defend against lipid peroxidation via different pathways.

Adding 1% whey protein hydrolyzate to the beverage has been shown to increase the antioxidant activity of the beverage (12). Mann et al. revealed that flavoured milk drinks fortified with 1% or 2% whey protein hydrolysate showed antioxidant activity (13). Contrary to this Garcia-Casas et al. reported that adding whey protein to a beverage rich in polyphenols with antioxidant

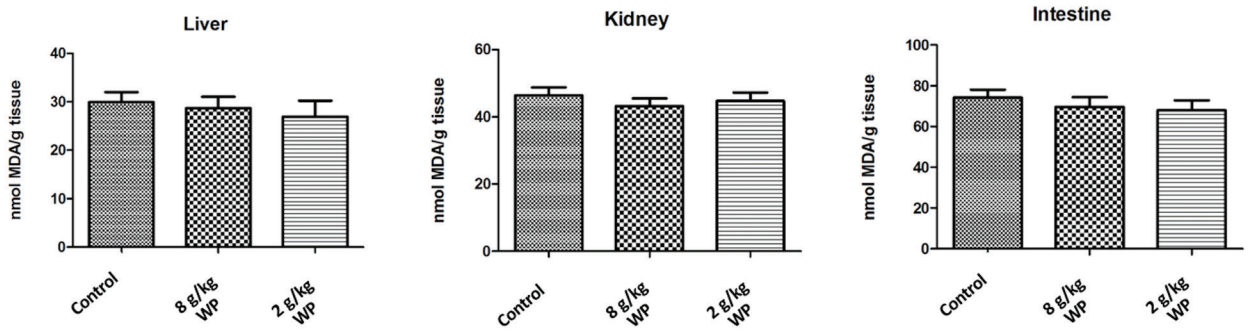

Figure 1: Malondialdehyde levels of liver, kidney and intestine tissues

MDA: Malondialdehyde, **WP:** Whey protein

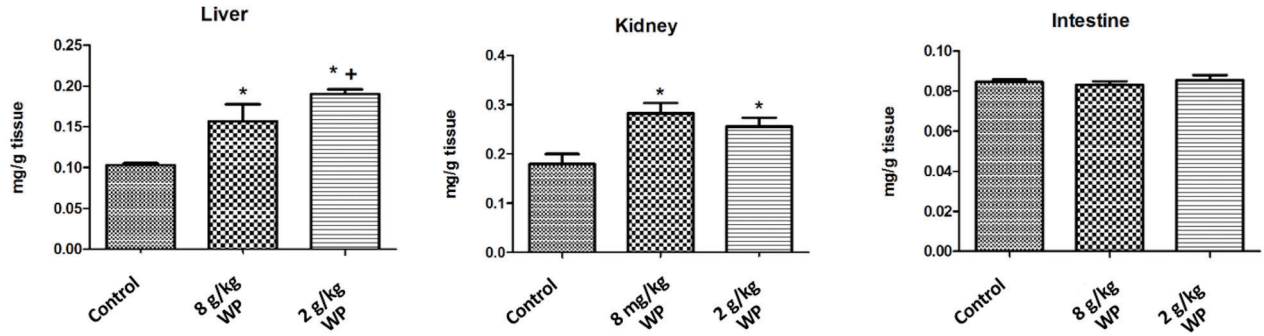

Figure 2: Glutathione levels of liver, kidney and intestine tissues

GSH: Glutathione, **WP:** Whey protein

*: p<0.05 compared to control group

+: p<0.05 compared to 8 mg/kg whey protein group

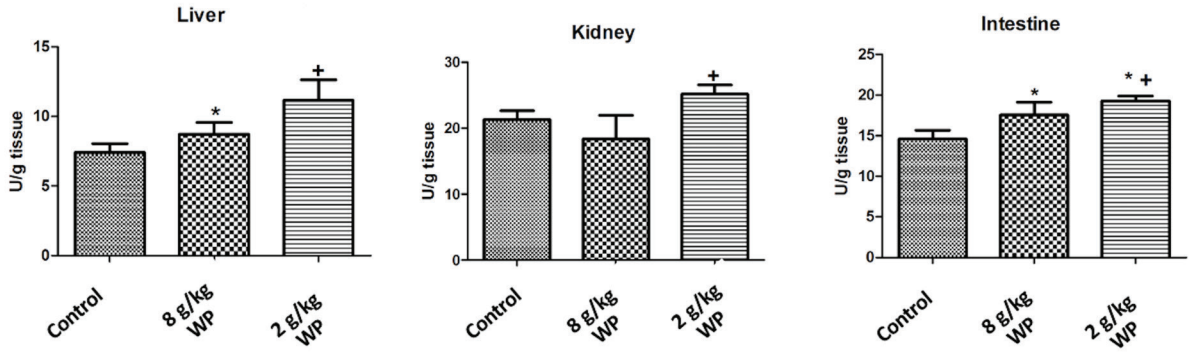

Figure 3: Superoxide dismutase activity of liver, kidney and intestine tissues

SOD: Superoxide dismutase, **WP:** Whey protein

*: p<0.05 compared to control group

+: p<0.05 compared to 8 mg/kg whey protein group

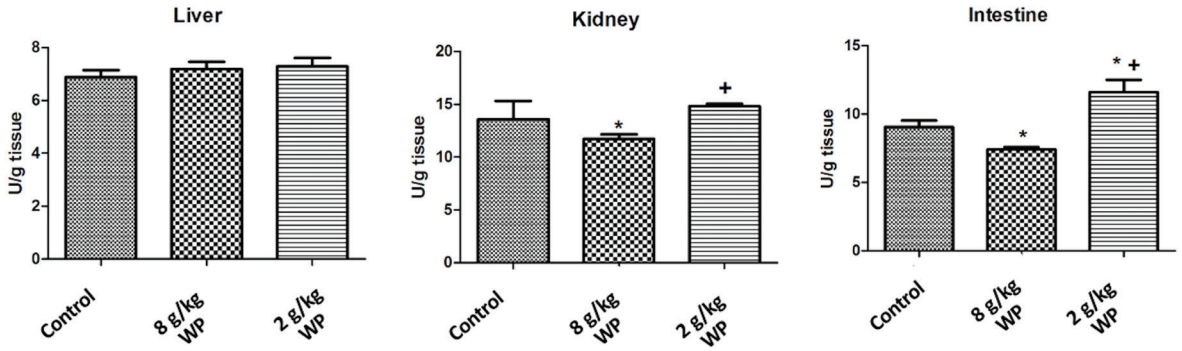

Figure 4: Glutathione-S-transferase activity of liver, kidney and intestine tissues

GST: Glutathion-S-transferase, **WP:** Whey protein

*: $p<0.05$ compared to control group

+: $p<0.05$ compared to 8 mg/kg whey protein group

properties does not create additional antioxidant activity (14). The optimal dosage of whey proteins that should be taken depends on the objectives of individuals, the amount of physical activity, and the composition of the body. However, some studies suggest that a dose of 20 to 25 g of whey protein per day is sufficient to achieve the proposed benefits, whereas doses above 40 g/day were associated with adverse effects on the organism (7). The optimal dose of whey proteins needs to be detected with experimental and scientific data. Experimental animal models and biochemical and histological examination of tissues may also provide valuable insights into the potential effects of whey protein administration.

Whey protein or its hydrolysates have been found to reduce lipid peroxidation caused by exercise, alcohol use, or diabetes (15). Furthermore, peptides derived from whey proteins have antioxidant activity as they can bind pro-oxidant metals or directly can scavenge free radicals (16). Whey protein's efficacy in preventing lipid peroxidation appears to be regulated by parameters such as concentration, degree of hydrolysis, and oxidative state. Since peptides with lower molecular weight can easily bind to lipids, it is hypothesized that they may have higher antioxidant potential (17).

In the present study, high dose whey protein administration did not increase lipid peroxidation in liver, kidney and intestinal tissues compared to the control group. While liver and kidney GSH levels increased with whey protein application, no change was detected in intestinal GSH levels. This result suggests that high doses of whey proteins are less effective on intestinal tissue compared to their effects on the liver and kidney. Liver and intestine SOD activities increased with both doses of whey proteins, but renal SDO activity did not change compared to the control group. Low-dose whey protein increased SOD activity in all tissues compared to high-dose whey protein. Liver GST activity did not change in all groups. Kidney and intestine GST activities decreased with the administration of high dose of whey protein compared to the control group. In low dose whey protein group, intestinal GST activity was also significantly higher than in the high-dose whey protein group.

5. Conclusion

In conclusion, a high dose of whey protein did not show the expected high antioxidant activity. It has

been determined that whey protein consumed at the optimum dose increases the antioxidant activity in the liver, kidney and intestinal tissue better than the high dose.

Conflict of Interest

The authors have no conflict of interest to disclose.

Ethics Approval

The experimental protocol of this study was approved by the Marmara University Animal Care and Use Committee (Approval date: May 26, 2021, Approval code: 502021.mar).

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