Protein Supplement Drinks, the Modern Killer that Induces Oxidative Stress in Mice Liver

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Abstract

Introduction: In past few years, the use of protein supplements is popular among body builders, as the strategy for weight control, and energy intake.

Aims: In the current study we aim to examine the effect of a protein supplement drink on oxidation-reduction systems in mice liver tissues.

Material and Methods: Twenty mice were randomly separated into two groups. The first experimental group; were feeding with a protein upplement and the other without protein supplement as a control groups over 12 weeks.

Results: The results highlighted that body weight was significantly lower (P =0.001) in the protein supplement group when compared with the second group. Additionally, we have observed significant differences in the level of malondialdehyde, total protein carbonyl, nitric oxide, and glutathione oxidized in liver tissue of the protein supplement group when compared with the control group.***Conclusion:*** Protein supplement drinks have a harmful toxic effect that can promote worse oxidative stress, especially on liver tissue, which could elevate the risk for the development of liver diseases in the long time.

Keywords*: protein supplement, oxidative stress, malondialdehyde, total carbonyl, nitric oxide, and glutathione*

Introduction

Protein molecules are performing a vast array of functions within the cells. Along with carbohydrates and fat, protein is one of the three macronutrients[1]. Constantly, Proteins are being degraded and synthesized in the body. Unlike carbohydrates and fats, amino acids are not stored in the body. Therefore, they should be gained from the diet, synthesized denovo, or produced by biodegradation of normal protein. The excess amino acids in the biosynthetic needs of cells are quickly degraded. As amino acids are oxidized, the nitrogen is converted to urea, uric acid, creatinine, and ammonia excreted by the kidneys [2]. The recommendation of protein intake for normal adults is 0.8 g/kg of body weight per day. Over the past few years, the consuming of protein supplement drinks (PSD) is gaining by body builders. Indeed, PSD is increasingly being recommended as one of weight control strategies. The protein supplement drinks appear to reduce body weight, appetite, energy intake, and improve plasma lipid profile. Despite the anti-obesity effects of PSD, the impact of such supplement on oxidative stress dose not examined [3,4].

During several metabolic reactions free radicals are generated, which contain unpaired electrons on outer orbital [5,6], and radicals can cause tissue damages as well as cell death because they are very reactive species [7].

When, the stringent balance between free radical generation and antioxidant defenses is disrupting the oxidative stress resulting, and that leads to undesirable damages for wide range of molecular species like lipids, proteins, and nucleic acids [8]. The cell-membrane polyunsaturated fatty acids are these targets of reactive oxygen species, in turn, lead to damage in the cell

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structure and function [9]. Also, lipid hydroperoxide decomposition leads to diversity of end products, such as malondialdehyde (MDA), which is now considered as a dependable marker of lipid peroxidation [10]. Additionally, total Protein carbonyl (TPC) are the products of the oxidative modification of proteins [11].

Also, Nitric oxide (NO) is a messenger molecule functioning immunity, in vascular regulation, and neurotransmission [12]. Previous researches have pointed the excess NO can play critical role in the altered pathophysiological mechanisms of diseases [13]. NO high concentrations are produced as a result of inducible nitric oxide synthase induction, and peroxynitrite formation [14].

While, anti- oxidant glutathione (GSH) has an important role in cascading of cellular defense versus oxidative injury. This molecule considered as the most available intracellular thiol-based antioxidant, that available in all living aerobic cells [15, 16].

Oxidative stress has been involved in the creation and complexity of diabetes mellitus, cancer as well as neurodegenerative diseases [17].

The aim of this study was to determine to what extent protein supplement drinks change the liver tissue content of total protein carbonyl, NO, MDA and GSH.

Materials and Methods

Animals and experimental design

A total of twenty albino male mice aged 2-3 months (22-30 g) were assigned into two groups (n=10), first ten fed with normal diet and the other fed with protein supplement drink (PSD) of whey protein. The cages were placed in a well-ventilated thermostatically regulated room (21±2ºC), relative humidity (40 to 60%), and (12:12) light-dark cycles. One week before to the experimental period, the mice were permitted for adaption to the experimental conditions. The experimental period lasted 4 weeks. The body weight of the animals was measured at the beginning of the experiment, and on the last day of the experiment. At the ending of the experimental interval, the mice were anesthetized with (ketamine-xylacine) and sacrificed. The internal organs (liver, spleen, and kidney) were extracted, washed in normal saline solution, then dried with a filter and weighed to calculate the relative organ weights (organ weight/body weight %). livers after weighing were immediately frozen in liquid N_2 and preserved at -80 ºC unto further analyses.

Experimental diet

Mice in the protein supplement drink (PSD) group were given protein supplement by oral feeding. The (Gold Standard 100% Whey) was purchased from a local market; PSD was weighted and fluxed in distilled water. The recommended consumption of Whey gold standard for humans is near (32g/ one intake). The mouse PSD dose (6.56 g/kg) used in this study was calculated according to Chen et al [18].

Chemical analyses

Liver homogenate samples for biochemical tests were collected and prepared according to the procedure provided by Elabscience colorimetric assay kits, and evaluated by the same assay kits for MDA, total protein carbonyl, NO and GSH.

Statistical Analysis

Statistical analysis: The data of the study were statistically analyzed using the statistical package for social science (SPSS) program (version 21, IBM Corporation, Somers, NY). The independent t-test analysis was carried out to compare between the mean values of the different parameters. Any two-tailed $P < 0.05$ was counted statistically significant result.

Results

The special effects of the protein supplement drink on the final body weight , relative weight of liver, kidney, and spleen of animals with PSD, and the control group, are shown in table 1.

The results highlight significant differences between the two groups on final body weight, relative weight of liver, kidney and the relative weight of spleen.

Table 1: Effects of the protein supplement drink on final body weight(g), the relative weight of liver, kidney, and spleen.

Parameter	Control group	PSD group	P value
Total Body weight (gm)	25.08 ± 1.84	21.9 ± 1.30	0.0003
Relative Liver weight (%)	5.15 ± 0. 1	5.24 ± 0. 2	0.7054
Relative Kidney weight (%)	1.31 ± 0. 26	0.64 ± 0.07	0.0231
Relative Spleen Weight (%)	0.71 ± 0. 18	1.39 ± 0.179	0.0150

Data are mean ± SEM, n = 10

Oxidative stress levels were determined through the assessment of oxidative end products of proteins (TPC), the oxidative end product lipids malondialdehyde (MDA), and nitric oxide (NO). All of the estimated oxidative damage products (TPC and MDA) were significantly higher ($P<0.0001$) in the PSD animals when compared to the control animals. While, there are a significant declination ($P<0.02$) in the concentration of glutathione reduced form in liver tissue of PSD animals when compared to the control animals, as in table 2.

Table 2: The levels of MDA, TPC, TNO, and GSH in liver mice tissues.

Parameter	Control group	PSD group	P value
MDA (ng/mg protein)	3.02 ± 0.41	5.98 ± 0.35	0.0001
TPC (µmol/mg protein)	29.06 ± 2.62	77.03 ± 2.09	0.0001
NO (µmol/g tissue)	0.106 ± 0.03	0.567 ± 0.01	0.0001
GSH (µg/mg protein)	9.82±2.15	3.57±1.99	0.0204

Results are expressed as mean ± SE. MDA, malondialdehyde. TPC, Total protein carbonyl. NO= (nitrite + nitrate), GSH, reduced glutathione.

Discussion

In the past few years, the use of protein supplements is popular among body builder, as a strategy for weight control, and energy intake [19]. Because the liver is particularly tending to oxidative damage and, due to the existence of many dialectical researches concerning its response to a high protein diet, we evaluated lipid and protein oxidation end products, and antioxidant status in liver tissues of mice fed a PSD. This is the first study that has compared oxidative stress induced by a chronic administration of PSD.

As a pointer of the toxicity of the PSD, we measured the changes in total body weight of mice, following 4 weeks. There were significant a difference in body weight loss when matched to the PSD group with their respective control group. In addition to that the relative organ weights were calculated. It is an important requirement to indicate the effect of xenobiotics present in PSD on certain organs between treated group and control group. So, the fresh weights of livers, kidneys, and spleen of mice were measured for recording of relative organ. There were no significant differences in the relative liver weights of mice fed with PSD compared to control group for 4 weeks. The differences were significant in

the relative weights kidneys and spleen of mice between PSD group and their respective control group after. Organs weight can be the most sensitive indicator of an experimental compound [20]. As a significant difference in organ weight between treated and control groups may occur. Actually, a compound that causes reduction in body weight and significant change in relative weights of most organs has a specific toxic effect on the organs. So, the results of the current study indicate that PSD has a harmful toxic effect.

Generally, the oxidative stress that caused by an imbalance between overproduction of reactive oxygen species and antioxidant defense [21,22]. In the current study, we have shown higher level of MDA, and TPC in liver tissue of PSD mice.

As proteins cannot be stored, high levels of protein intake lead to elevated amino acid oxidation to keep the homeostasis of amino acid in the body. The re-oxidation of reducing equivalents generated from amino acids oxidation can raise the free radical formation, via electron flow along the mitochondrial respiratory chain [23, 24].

The existence of free radicals has some harmful effects on several sensitive molecular and cellular components such as proteins, and membrane lipids [25]. As mentioned above the cell-membrane polyunsaturated fatty acids are the primary targets of reactive oxygen species, which, in turn,can lead to many damages in the cell structure and function [26].

The results of our study, suggest that PSD consumption used in the present study increases the levels of MDA, and TPC in liver tissue. The levels of MDA, and TPC in the liver are considered especially sensitive to oxidative stress results from PSD. The current study, also detected a significantly lower levels of GSH in the liver tissues of animals with PSD in respect to control group liver tissues. It is recognized that GSH is the most vital component of antioxidant tissue system defense and the only protective compound that can scavenges the hydroxyl radicals [27].

Previously it was confirmed that lowered levels of GSH lead to mitochondrial damage in the liver [28]. So, the reduced GSH levels that detected in this study can be a proof for the increasingly oxidative damage in liver despite the fact that enzymatic antioxidants were markedly increased.

In our research, we observe a significant change in the liver NO levels within the group feeding with PSD, possibly due to the difference between the liver cells metabolism and/or regulation of NO in them. A reduced release of NO is one of the classical markers of endothelial dysfunction. Also NO is decreasing in rats with chronic renal failure [29].

In conclusion, the data of this study suggests that the consumption of protein supplements may induce oxidative stress in the liver tissue of mice. So, further studies will be necessary to understand the mechanisms of PSD on other tissues.

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Conflicts of interest: Declared none.

Ethics Statement: This experiment was approved by the Central Committee for Bioethics in college of Sciences/ Kufa, Iraq.

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