ACUTE EFFECTS OF WHEY PROTEIN and VITAMIN C COMBINATION ON THE HEMODYNAMICS OF THE MALE STUDENTS

by

Hatice Dilek ULUSAN

THESIS SUBMITTED TO
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE ABANT IZZET BAYSAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
IN
THE DEPARTMENT OF BIOLOGY

DECEMBER 2008

Approval of the Graduate School of Natural and Applied Science.

Prof. Dr. Nihat ÇELEBİ
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of the Master of Science.

Prof. Dr. Ömer BOZDOĞAN
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Biology.

Assist. Prof. Dr. Bekir YÜKTAŞIR
Co- Supervisor

Assist. Prof. Dr. Seyhun YURDUGÜL
Supervisor

Examining Committee Members

1. Prof. Dr. Faruk BOZOĞLU .....

2. Prof. Dr. Ömer BOZDOĞAN .....

3. Assist. Prof. Dr. Seyhun YURDUGÜL .....

ABSTRACT

ACUTE EFFECTS OF WHEY PROTEIN and VITAMIN C COMBINATION ON THE HEMODYNAMICS OF THE MALE STUDENTS

Ulusan, Hatice Dilek
Master of Science, Department of Biology
Supervisor: Assist. Prof. Dr. Seyhun YURDUGÜL
Co-Supervisor: Assist. Prof. Dr. Bekir YÜKTAŞIR

December 2008, 58 pages

Whey protein is a rich source of biologically active molecules that are able to influence a range of physiological functions in the organism. Whey protein has the ability to act as an antioxidant, antihypertensive, antitumor, antiviral, antibacterial, hypolipidemic and immune modulating agent.

On the other hand, vitamin C is a highly effective antioxidant which protects the body against oxidative stress. It protects the immune system by stimulating the activity of antibody and immune system cells such as neutrophils.

This study aimed to investigate the acute effects of whey protein and vitamin C combination on the hemodynamics of the male students. Forty healthy volunteers were participated and this study was carried out as double blind and randomized. The volunteers were randomly assigned to one of four groups (n=10 for each group). The first group was the control or the placebo group (CG). The second group was treated with the whey protein (WPG)(30 g). The third group was treated with the vitamin C (VCG) (1000 mg). The fourth group was treated with the combination of vitamin C

and whey protein (WCG). The blood samples were obtained prior to supplementation (pre-treatment) and after 75 minutes of ingestion of the related supplementation (post-treatment). Hematological data was obtained and the pH of blood were determined.

As a result; no difference between the groups were observed. In the group treated with whey protein and vitamin C (WCG), the number of granulocytes was significantly higher in the post- treatment than the pre-treatment. The pH of blood did not vary between the groups according to the statistical evaluation (*$p>0.05$).

Keywords: Whey protein, Vitamin C, Hematological parameters, Blood

ÖZET

AKUT OLARAK PEYNİRALTI SUYU PROTEİNİ VE VİTAMİN C'NİN BİRLİKTE ALIMININ ERKEK ÖĞRENCİLERDEKİ KAN PARAMETRELERİNE ETKİLERİ

Ulusan, Hatice Dilek
Yüksek Lisans, Biyoloji Bölümü
Tez Danışmanı: Yard. Doç. Dr. Seyhun YURDUGÜL
Yardımcı Tez Danışmanı: Yard. Doç. Dr. Bekir YÜKTAŞIR

Aralık 2008, 58 sayfa

Whey protein (Peyniraltı suyu proteini) organizmada bir dizi fizyolojik fonksiyonu etkileyen zengin bir protein kaynağıdır. Peyniraltı suyu proteini, antioksidan, antihipertansif, antitümör, hipolipidemik, antiviral, antibakteriyel ve bağışıklık düzenleyici özelliklere de sahiptir. Çok etkili bir antioksidan olan vitamin C vücudu oksidatif strese karşı korur. Antikor aktivitesini ve bağışıklık hücrelerini uyararak bağışıklık sistemini korur.

Bu çalışmada amaç, akut olarak peyniraltı suyu proteini ve vitamin C'nin birlikte alımının erkek öğrencilerdeki kan değerlerine etkisini araştırmaktır. Bu çalışma deneyden habersiz ve rasgele seçilmiş 40 gönüllü katılımcı ile yürütülmüştür. Katılımcılar rasgele dört gruba ayrıldıktan sonra ilk olarak katılımcılardan (katılımcı sayısı her grup için 10 kişi) kan örnekleri alınmış ve sonra birinci gruba kontrol çözeltisi (plasebo), ikinci gruba peyniraltı suyu proteini (30 g), üçüncü gruba vitamin C (1000 mg) ve dördüncü gruba ise peyniraltı suyu proteini ve

vitamin C birlikte verilip 75 dakika sonra tekrar kan örnekleri alınarak, hematolojik değerler ve kan pH'ları üzerine çalışılmıştır.

Sonuç olarak, gruplar arasında farklılık gözlenmemiştir. Peyniraltı suyu proteini ve vitamin C' yi birlikte alan grupta, granülosit değerlerinde anlamlı bir artış gözlenmiş olup kan pH' larında önemli bir değişiklik gözlenmemiştir (*p>0.05).

Anahtar Kelimeler: Peyniraltı suyu proteini, Vitamin C, Hematolojik parametreler, Kan.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor**,** Assist. Prof. Dr. Seyhun YURDUGÜL, for his support and suggestions in the completion of this thesis. I would also like to thank to my co-supervisor, Assist. Prof. Dr. Bekir YÜKTAŞIR, who has advised and guided me throughout this research. I thank to Doç. Dr. Muzaffer DÜGEL for his support.

I offer my special thanks to Res. Assist. Canan SAPMAZ, Züleyha AYGÜN and Res. Assist. G.Gökçe YILDIZ for support and for their endless friendship.

I would like to thank to Abant İzzet Baysal University Presidency Health Culture and Sports Department for technical support in this study. I also acknowledge to the volunteers who participated in this study.

Finally, I am grateful to my family for their encouragement, support and love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BCAA : **B**ranched **C**hain **A**mino **A**cid

CG : **C**ontrol **G**roup

DHAA : **D**e**h**ydro-L-**A**scorbic **A**cid

GRAN : **G**ranulocytes **(K/µL)**

HCT : **H**ematocrit **(%)**

HGB : **H**emoglobin **(g/dL)**

LYM : **L**ymphocytes **(K/µL)**

MCH : **M**ean **C**ell **H**emoglobin **(pg)**

MCHC : **M**ean **C**orpuscular **H**emoglobin **C**oncentration **(g/dL)**

MCV : **M**ean **C**orpuscular **V**olume **(fL)**

MID : Precursors of White Blood Cell **(K/µL)**

PLT : **P**latelets **(K/µL)**

RBC : **R**ed **B**lood **C**ell **(M/µL)**

RDW : **R**ed Blood Cell Volume **D**istribution **W**idth **(%)**

ROS : **R**eactive **O**xygen **S**pecies

VCG : **V**itamin **C** **G**roup

WBC : **W**hite **B**lood **C**ell **(K/µL)**

WCG : **W**hey **P**rotein **G**roup

WPG : **W**hey **P**rotein **G**roup

1 INTRODUCTION

Milk constituents have become recognized as functional food. Whey protein, a by - product of cheese and a protein complex derived from milk, is comprised of high protein content that proteins have different physical functions (Ha and Zemel, 2003).

The discovery of whey protein as a functional food with nutritional application elevated whey to a co-product in the manufacturing cheese. Historically, whey was considered a cure, used to heal ailments ranging from gastrointestinal complaints to joint and ligament problems. Nanna Rognvaldardottir, who is an Icelandic food expert, describes how whey, called syra by the Icelandic people, is fermented and stored in barrels. Syra is diluted with water and ingested or used as a marinade or preservative for meat and other food. So, syra was the most common beverage of Icelandic people (Marshall, 2004).

Whey is the soluble fraction in milk. Milk contains two primary sources of protein, the casein and whey. Caseins are the proteins responsible for making curds, while whey remains in an aqueous environment. A number of whey products vary with respect to concentrations of proteins, minerals, lipids, and lactose (Fuente et al., 2002; Considine et al., 2007).

Whey proteins are now widely used in food as gelling agents, emulsifiers, texture modifiers, thickening agents and foaming agent. Commercial whey protein are considered as ‘generally recognized as safe’ (GRAS) substances for food product applications, making them “label friendly” ingredients. Whey protein can be added to special foods to increase value (Ferreira et al., 2007; Bryant and McClements, 1998).

1.1 Whey Protein Manufacturing

When casein is removed from whole milk, liquid whey remains. Milk is pasteurized by the high temperature-short time method (163 degree F for 20 second) and held overnight at 40 ^{o}C. Then, the mixture is cooled to 30 ^{o}C, inoculated with a lactic acid culture and incubated for 30 minutes. Rennet extract is added, resulting in the coagulation of curd, the liquid whey is drained through a stainless steel screen. Whey liquid is filtered at 45 ^{o}C and brought to a pH of 3 by adding citric acid. This can be micro-filtrated to increase protein concentration. Difiltration is used to remove most of the lactose, minerals and other low molecular weight components. Ion-exchange process is used to remove fat and lactose. Finally, whey protein concentrate is warmed and spray-dried to achieve whey protein powder (Fuente et al., 2002; Marshall, 2004).

1.2 The Functional Properties of Whey Protein

Whey and whey derived bioactive compounds have important functional properties and enhance the general health. Whey has potent antioxidant activity, antimicrobial activity, improves the muscle strength and body composition, helps in the immune modulation, prevent cardiovascular disease and osteoporosis (Marshall, 2004; Ha and Zemel, 2003).

Whey protein is best aminoacid source for athletes or exercising humans. Reactive oxygen species (ROS) are the physiological products of aerobic metabolism. Exercise is associated with an increased ATP requirement and an enhanced aerobic or anaerobic metabolism, which increased the formation of ROS (Radak et al., 2007). Whey protein contains 2,5% cysteine which is the richest dietary source of the glutathionine precursors. The precursor promotes to increase cellular glutathione level, major intracellular oxidant, that are reduced by physical

activity (Colker et al., 2000). Endogenous oxidant system, (GSH) glutathione (glycine, cysteine, glutamic acid) defends against excess ROS accumulation in body (Rankin et al., 2006; Blouet et al., 2007). ROS are also believed to be involved in pathological process such as, atherosclerosis, cancer and neurodegenerative diseases (Alzheimer, Parkinson, inflammation, rheumatic arthritis, ischemia) (Radak et al., 2007).

Whey protein contains relatively a high proportion of branched- chain amino acid (BCAA ~ 26%). The branched chain aminoacids are leucine, isoleucine, valine. Particularly, leucine plays a distinct role in protein metabolism and has been identified as a key signal in the translation initiation pathways of the muscle protein. Leucine have anabolic effects on protein metabolism by increasing the degradation in resting human muscle (Blomstrand et al., 2006; Ha and Zemel, 2003). BCAAs suppress muscle protein catabolism when ingested orally within 4 hours of exercise. Oral BCAA administration appears to prevent muscle loss during times of stress, slows the degradation of glycogen during prolonged submaximal exercise (Colker et al., 2000).

The consumption of whey protein decreases the level of blood lactate after exhaustion and increases the concentration of serum albumin (Pimenta et al., 2006), thus intake of whey protein may increase its efficiency in terms of physical performance (Tipton et al., 2006). Paddon- Jones et al. showed that acute ingestion of amino acid stimulates muscle protein synthesis (Paddon-Jones et al., 2005).

Whey contains a number of immunomodulatory peptides which are naturally present. Several research showed that whey protein modulates the proliferation of lymphocytes in vitro and in vivo. Bovine lactoferrin which is the component of the whey protein stimulate the release of neutrophil-activating IL -8 (interleukin–8) from

the human polymorphonuclear leukocytes and promotes the phagocytic activity of human neutrophils. Glycomacropeptide which is a component of the whey protein induces the production of IL -1(interleukin -1) family cytokines in macrophage cell lines and enhances the proliferation and phagocytic activity of human macrophage. Lactoferrin has antimicrobial, antifungal, antiviral activity (Mong et al., 1997; Mercier et al., 2004; Gauthier et al., 2006).

Whey is reported to reduce the blood pressure. Antihypertensive peptide which is isolated in the primary sequence of bovine β-lactoglobin, gives the whey a significant angiotensinogen I-converting enzyme (ACE) inhibitory activity, which blocks the conversion of angiotensin I to angiotensin II (Ferreira et al., 2007).

Whey has an important role on suppressing hepatic fatty acid synthesis, decreasing accumulation of the body fat and preventing blood serum cholesterol to rise (Morifiji et al., 2005).

Whey protein has antitumor and anticarcinogenic properties. Smithers et al showed that the rats fed with whey protein displayed the lowest incidence of colon cancer, that is, the rats fed whey protein showed a statistically significant reduction in the total numbers of tumors (Smithers et al., 1996).

Whey protein has a protective effect on the gastric mucosa. Glycomacropeptide in the whey exhibit prebiotic or probiotic activity and stimulate the inhibition of toxin binding (Ha and Zemel, 2003).

1.3 The Absorption of Whey Protein

The absorption of whey protein is faster than the absorption of casein. Because whey protein is a soluble protein whereas casein clots into the stomach. Thus, casein delay in the stomach, results in a slower release of aminoacid. Whey

protein is considered to be “fast protein” as it reaches the jejenum quickly after entering the gastrointestinal tract (Boirie et al., 1997; Marshall, 2004).

1.4 The Adverse Effect of Whey Protein

No severe adverse reaction have been noted following the administration of whey protein products, although some patients note some minor gastrointestinal disturbances. Most whey proteins are processed to remove lactose. It contains trace amount of lactose, so, whey protein is reported to be suitable for lactose intolerant people (Marshall, 2004).

1.5 The Biological Components of Whey Protein

Whey protein is comprised of various amount of proteins such as β-lactoglobin, α-lactoalbumin, serum albumin, lactoperoxidase, lactoferrin and many growth factors such as the epidermal growth factor, the interleukins, insulin like growth factor I, II, glycomacropeptides and the immunoglobulins. Whey protein has a high concentration of branched - chain aminoacids (leucine, isoleucine, valine) and the sulfur containing aminoacids (cysteine, methionine) (Smithers et al., 1996; Mong et al., 1997; Mercier et al., 2004).

1.5.1 Lactoferrin

Lactoferrin is a non-heme iron binding glycoprotein that consists of a single polypeptide chain of 78 kDa molecular weight. It is found in most of the exocrine secretions including the tears, nasal secretion, saliva, intestinal mucosa and genital secretion, in epithelial secretion, in the secondary granules of neutrophils (Conneely, 2001).

Lactoferrin has antimicrobial, antifungal, antiviral, antiparasitic and antitumor activities (Zimecki and Artym, 2005). A potent antimicrobial peptide,

'lactoferricin' is generated upon the gastric pepsin cleavage of lactoferrin. The active peptide consists of a loop of 18 aminoacid residues (Tomita et al., 1994).

Human lactoferrin, bovine lactoferrin, and lactoferricin stimulate the release of neutrophil-activating IL-8 from the human polymorphonuclear leukocytes and the phagocytic activity of human neutrophils (Gauthier et al., 2006).

Lactoferrin increases the number and the activity of T and B lymphocytes and nature killer cells which are a type of cytotoxic lymphocyte (Audesirk et al., 2002), the phagocytic activity and cytotoxicity of monocytes/macrophage, stimulates the release of number of cytokines (IL–1, IL–6, IFN-gamma, TNF-α) (Artym, 2006).

1.5.2 β – Lactoglobulin

β-lactoglobulin is the most important protein in whey, approximately 45% of the total whey protein is β-lactoglobulin (Heddleson and Alen, 1997). β-lactoglobulin is a small, globular protein that folds into an 8-stranded, antiparallel β-barrel with a 3-turn α-helix on the other surface. β-lactoglobulin, a carrier of small hydrophobic molecules including retionic acid, binds both fatty acid and retinol (Kontopidis et al., 2004). The human milk contains no beta-lactoglobulin. β-lactoglobulin is a rich source of cysteine which is essential for the aminoacid stimulate glutathione synthesis (de Wit, 1998).

1.5.3 α - Lactoalbumin

α- lactalbumin, is one of the main proteins found in human and bovine milk. α- lactalbumin is stabilized by four disulphide bonds having a molar mass of 14.2 kDa and has no free thiol group (Considine et al., 2007). Approximately 20 -25 percent of whey protein is α- lactalbumin (Marshall, 2004). α- Lactalbumin has a calcium binding site which stabilizes its native configuration, and the ability of α-

lactalbumin to bind minerals (Calcium and Zinc) may positively affect their absorption (Kelleher et al., 2003). One of the biological activities of both α-lactalbumin and β-lactoglobulin is the inhibition of angiotensin – converting enzyme (ACE) (Ferreira et al., 2007).

1.5.4 Bovine Serum Albumin

Bovine serum albumin is reported to be a large protein which is one of the main sources of essential aminoacids, comprises approximately 10 -15 percent of total whey protein (Marshall, 2004). Bovine serum albumin is a single polypeptide of 582 aminoacid residues with a molecular weight of 66.433 Da and exists in a multidomain structure with complex ligand-binding specificities (Considine et al., 2007). Bovine serum albumin binds insoluble free fatty acids for transportation in the blood (de Wit, 1998).

1.5.5 Glycomacropeptide

Glycomacropeptide, a casein macropeptide, is composed of a peptide containing 64 aminoacids, is present about 10 -15 percent in whey and it contains no aromatic compounds (phenylalanine, tryptophan and tyrosine) (Aimutis, 2004). Glycomacropeptide has various biological activities, including binding to cholera toxin and *Escherichia coli* enterotoxins, suppression of the gastric secretion, modulation of the immune response, the promotion of Bifidobacterial growth and the inhibition of the bacterial and viral adhesion. The other properties of glycomacropeptide are providing good palatability and functional properties imparting favorable mouth-feel and flavor to foods (Mikkelsen et al., 2006).

1.5.6 Lactoperoxidase

Whey protein contains various enzymes, including the hydrolases, transferases, lyases, proteases and lipases. Lactoperoxidase, the most abundant enzyme, is present at 0.25 -0,5 percent of total protein in whey (Marshall, 2004). Lactoperoxidase is present in tears, saliva, and milk. Lactoperoxidase utilizes H_2O_2 (hydrogen peroxide) to oxidize thiocyanate to hypothiocyanate which is an antibiotic compound that prevents the growth of bacteria, fungi and viruses (Rather and Prince, 1999).

1.5.7 Immunoglobulins

An immunoglobulin (Ig) is an antibody or gamma-globulin. In an adult, approximately 75 percent of the antibodies is IgG. The other immunoglobulins are IgM, IgD, IgA, IgE. IgG is transported from mother to child via cord blood and by breast-feeding. This refers to passive ‘immunity’. Approximately 10–15 percent of total whey protein is immunoglobulins (de Wit, 1998).

1.5.8 The Growth Factors

Proteins in whey serve as potent growth stimulants for a number of mammalian cell lines in culture. These growth factors have an important impact on cell growth by promoting synthesis of DNA and protein and by inhibiting the degradation of protein. The growth factors are IGF-I, IGF-II, platelet derived factor, acidic and basic fibroblast growth factor and the transforming growth factor-β (Smithers et al., 1996).

1.6 The Essential Aminoacids in Whey Protein

Leucine, lysine, valine, isoleucine, threonine, methionine, phenylalanine, trytophan are found in whey protein (Marshall, 2004).

1.7 The Non - Essential Aminoacids in Whey Protein

Arginine, cysteine, aspartic acid, glycine, alanine, glutamic acid, serine, histidine, proline, tyrosine are found in whey protein (Marshall, 2004)

1.8 Blood Cells

Whole blood consists of erythrocytes (red blood cell), leukocytes (white blood cell), trombocytes (platelets) and plasma (i.e. fluid). Hematopoiesis is the production of blood. All of the blood cell arise from the pluripotent stem cell, which is under the influence of hematopoietic stem cell (Steven, 1997).

The mature red blood cell, the erythrocyte, transports O_2 to tissues and CO_2 to lungs from tissues. (Audesirk et al., 2002; Bozdoğan, 2004). Leukocytes refers to white blood cells (WBC) including the granulocytes (neutrophils, eosinophils and basophils), monocytes/ macrophages and lymphocytes/ plasma cells, responsible for defending the body from the invasion and the infection (Steven, 1997). The granulocytes (neutrophils, eosinophils and basophils) and monocytes contain granules in the cytoplasm and are produced in the bone marrow. The neutrophils are the major component of the granulocytes having highly potent bactericidal and fungicidal properties (Nagatomi, 2006). The eosinophils, classified in the granulocytes have low phagocytic activity and their number increase in the allergy, anaphylaxis and parasitic diseases. The basophils have less or no phagocytic activity and responsible of secreting heparin, histamin, bradykinin, serotonin and the lysosomal enzymes during inflammation (Bozdoğan, 2004). Lymphocytes play an

important and integral role in the body's defense (Schmaier and Petruzzell, 2003). Most, but not all large granular lymphocytes are more commonly known as the natural killer cells. The small lymphocytes are the T-cell and B-cells. (Tkachuck, Hirschman and McArthur, 2001). T-cells are involved in cell- mediated immunity whereas B-cells are responsible for humoral immunity (correlating to antibodies) (Bozdoğan, 2004). The platelets (PLT) have an important role on hemostasis and able to repair vascular openings. At the site of any rent in a blood vessel wall elicit activation of platelet and PLT can stop blood loss (Andrews et al., 1997; Marcus and Safier, 1993).

1.9 Hematological Parameters

1.9.1 The Hemoglobin (HGB)

The hemoglobin is the major protein in RBC. A hemoglobin molecule consists of four globin chains. Each globin chain is bound to a heme moiety containing iron (Schmaier and Petruzzell, 2003). Blood hemoglobin concentration value gives information about osmotic dynamics in plasma, blood viscosity and oxygen carrying capacity (Andrijauskas and Iuaskeuicius, 2006).

1.9.2 The Hematocrit (HCT)

The viscosity of blood is important for body function. The blood viscosity is especially influenced by RBC and protein concentration and the RBC size. The hematocrit value provides information relative to the percentage of red blood cell in whole blood (Schmaier and Petruzzell, 2003). The hemoglobin and hematocrit values are related to nutritional supplementation such as protein, iron etc..(Edozien and Switzer, 1977).

1.9.3 The Mean Corpuscular Volume (MCV)

The MCV reflect the volume of the size of the red blood cell. MCV falls in microcytic (small cell) anemia and rises in macrocytic anemia. The normal reference range is 80 to 95 femtoliters (Stevens, 1997).

1.9.4 Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC is the concentration of hemoglobin in each red blood cell and is calculated by dividing the hemoglobin by the hematocrit. The normal reference range is 32 to 36% (Stevens, 1997). Edozien and Switzer showed that the hemoglobin concentration, hematocrit and MCHC are very sensitive to level of protein intake (Edozien and Switzer, 1977).

1.9.5 The Mean Cell Hemoglobin (MCH)

The MCH refers to the mass of the red blood cells and calculated by dividing the hemoglobin by RBC count. The normal reference range is 27 to 31 picograms (pg) (Schmaier and Petruzzell, 2003).

1.9.6 The Red Blood Cell Volume Distribution Width (RDW)

The RDW describes the heterogenecity in RBC size with blood sample. The normal range is 11.5–14.5%. In patient with iron deficiency, an increase in RDW value were observed. The RDW is useful in the subclassification of anemia when used in conjugation with the MCV (Park and Kim, 1987).

1.10 Vitamin C

Vitamin C, ascorbic acid, potent antioxidant, is water soluble vitamin, hydrophilic antioxidant in biological fluids, accumulates in the cytosol and extracellular fluid (Thamson and McNaughton, 2001).

The name ascorbic acid comes from word ‘anti-scurvy’ acid. The ascorbic acid or vitamin C was discovered after scientists had searched for centuries for a drug to curve the disease known as scurvy which was caused by deficiency of vitamin C. The Scottist naval surgeon James Lind found that men suffering from scurvy were cured when given oranges and lemons and he published his findings in the Treatise on Scurvy in 1753 (Padayatty and Levine, 2001; Paasch and Salzer, 2004).

Hungarian scientist Albert Szent-Györgyi isolated the ascorbic acid from the orange juice, cabbage juice and adrenal gland of oxen in 1928. This compound was identified as vitamin C, antiscorbutic substance by Waugh and Kind in 1932, and synthesized by van Reichstein in 1933 that the chemical picture was complete (Vilter, 1980).

1.10.1 The Biochemistry of Vitamin C

Vitamin C (ascorbic acid, ascorbate) is a six-carbon-lactone $C_6H_6O_8$. Molecular mass of vitamin C is 176,13 g/mol. Vitamin C is the L-enantiomer of the ascorbate, the opposite D-enantiomer has no physiological significance. Both forms are the mirror image of the same molecular structure. L- ascorbate is a strong reducing agent carries out its reducting function. Vitamin C is not a soluble organic compound (Kırıkoğlu, 2002).

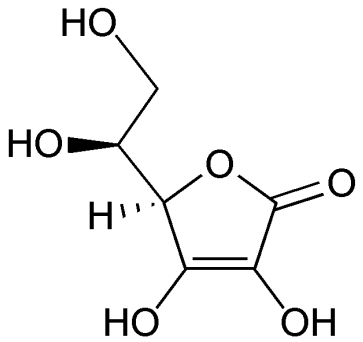


Figure 1 The Structure of Vitamin C (URL 1)

Vitamin C is an electron donor and a reducing agent. Thus, all known biochemical and physiological action of vitamin C are due to its function as a reducing agent (Levine et al., 1999).

The vitamin C is the least stable of the vitamins and readily destroyed by exposure to air and its degradation increases by exposure to heat, alkali and metals. The cooking of vegetables and fruits can decrease their vitamin C content by 20% to 40% (Hampl et al., 2004).

1.10.2 The Synthesis of Vitamin C

Vitamin C is synthesized from glucose in the liver of most mammalian species, but humans, non-human primats and guinea pigs, some birds are not able to synthesize vitamin C. These species do not have the enzyme gulonolactone oxidase. This enzyme is essential for synthesis of ascorbic acid immediate precursor 2-keto-1-gulonolactone (Padayatty et al., 2003). Humans must intake vitamin C to survive (Thompson and McNaughtan, 2001).

1.10.3 The Source of Vitamin C

Vitamin C is found in many fruits and vegetables. Rich fruit sources include grapefruit, honeydew, kiwi, orange, mango, papaya, strawberries, tangelo, tangerine, watermelon. Fruit juice including vitamin C are grapefruit and orange juice. Rich vegetable source of vitamin C are asparagus, broccoli, cabbage, cauliflower, kale, mustard greens, pepper, potatoes, snow peas, tomato juices (Padayatty et al., 2003). The vitamin C content in fruits and vegetables is related to harvesting season, duration of transport to the marketplace, cooking practices, period of storage and shelftime (Levine et al., 1999).

1.10.4 The Absorption of Vitamin C

Vitamin C is readily absorbed in stomach and small intestine. Vitamin C concentration in plasma are controlled as a function of dose. At plasma concentration less than 4 μm, symptoms of scurvy may occur. The control of vitamin C concentration is mediated by tissue transport, absorption and excretion (Padayatty et al., 2003). In the intestine, absorption of vitamin C is inversely related to dose. If higher doses are administered, less absorption arises. At a dose of 30 mg, approximately 90 percent of the dose is absorbed. At a dose of 1250 mg, less than 50 percent of the dose is absorbed. Thus, dose-concentration relationship is sigmoidial (Padayatty and Levine, 2001).

Ascorbic acid is transported into the cell by sodium-dependent vitamin C transporter SVCT2, one or both of which are localized in most tissues (Padayatty and Levine, 2001). Vitamin C is actively co-transported with sodium against an electrochemical gradient into intestinal epithelial cells (Bsoul and Terezhalmy, 2004).

In leukocytes, vitamin C is passed into cell by means of passive diffusion. In trombocytes, adrenal and retinal cells, vitamin C is actively transported into cell. Vitamin C which is absorbed in intestine, comes to liver and then transported to the part of body (Kırıkoğlu, 2002).

In other words; about 80–90% ascorbic acid is absorbed in the gastrointestinal tract. The absorbed vitamin C circulates freely in plasma, leukocytes and red blood cell and enters all of the tissues. The body uses it in two hours and then usually out of the blood within three to four hours. Vitamin C is used in more rapidly under stressful conditions (Iqbal et al.,2004).

At the intestine and cell, ascorbic acid is oxidized to dehydro-L-ascorbic acid (DHAA) which is more quickly transported across the cell membrane. Once inside the tissue or intestinal epithelium, the DHAA is reduced to ascorbate. Facilitated diffusion of ascorbate into the circulation is sodium independent and follows a concentration and electrochemical gradient (Boroul and Terezhalmy, 2007).

Some of dehydroascorbic acid is metabolized by hydrolysis and is lost. If dehydroascorbic acid is not reduced back to ascorbic acid, DHAA is hydrolyzed irreversible to 2,3 diketogulonic acid. This molecule is metabolized into xylose, xylonate, lyxonate and oxalate (Padayatty et al., 2003).

1.10.5 The Excretion of Vitamin C

Vitamin C undergoes glomerular filtration and renal absorption. Ascorbate probably transmit unchanged through glomeruli and undergoes concentration-dependent active tubular reabsorbtion by vitamin C transport protein. When the saturation of the transport protein (achieves V_{max}) occurs, remaining vitamin C is not transported and is excreted in urine (Levine et al., 1999).

1.10.6 The Recommended Dietary Allowance

The current recommended dietary allowance (RDA) for vitamin C is 60 mg day. The RDA was based on preventing the scurvy on threshold urinary excretion of vitamin, on estimates of vitamin C absorption, rates of depletion, turnover and catabolism. The recommendations for vitamin C intake are under revision by the Food and Nutrition Board of the National Academy of Science (Levine et al., 1996; Levine et al., 1999). The current RDA for smokers is 110 -125 mg/day. The current RDA for women during pregnancy and lactation is 85 and 120 mg/day, respectively (Bsoul et al., 2004).

Mega doses of vitamin C is used in the treatment and prevention of large number of disorders like diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart diseases and cancer (Iqbal et al., 2004).

1.10.7 The Role of Vitamin C

Vitamin C, a potent antioxidant, protects both cytosolic and membrane component of cell from oxidative damage of free radicals. In cytosol, ascorbate acts as a primary antioxidant to scavenge free radical due to its chemical properties (May, 1999).

Vitamin C can enhance the body's resistance to various diseases. It protects the immune system by stimulating the activity of antibody and immune system cell such as neutrophils. Test tube studies showed that vitamin C stimulates phagocytosis. Vitamin C helps the immune system to fight viruses and acts as an antiviral agent (Iqbal et al., 2004; Bsoul et al., 2004). Vitamin C has an important role as a cofactor for 8 enzymes involving in collagen hydroxylation, biosynthesis of carnitine and norepinephrine from dopamin, amidation of peptide hormones, tyrosine metabolism. Vitamin C also has many non-enzymatic actions (Padayatty and Levine, 2001).

Vitamin C contributes to the synthesis of the amino acid carnitine and the catecholamines that regulate the nervous system. It helps to break down histamine, the inflammatory component of many allergic reactions (Iqbal et al., 2004).

The major carrier of the cholesterol and the triglycerides in plasma is the low-density lipoprotein (LDL). Oxidized LDL is atherogenic after structural modification. Vitamin C prevents the oxidation of the LDL (Levine et al., 1999).

The role of vitamin C in the synthesis of collagen is to promote the formation of hydroxyproline. Nonhydroxylated collagen is unstable and can not form the triple

helix required for normal structure of subcutaneous tissue, cartilage bone and the teeth (Bsoul et al., 2007).

Vitamin C increases iron absorption from the small intestine and regulates the iron distribution and storage. In addition, vitamin C may increase the endothelial nitric oxide (NO) by protecting it from oxidation (Padayatty et al., 2003). Thus, vitamin C has beneficial effects on the vascular endothelial function and cardiovascular disease (Silvestro et al., 2003), the treatment of common cold age-related eye diseases, inhibition of the bacteria, enhancing the mobility of polymorphonuclear (PMN) leukocytes (Bsoul et al., 2004).

The cellular uptake of vitamin C is promoted by insulin and is inhibited by hyperglycemia. In the absence of insulin, hyperglycemia produces “tissue scurvy”. The complication of diabetes mellitus are believed to result from either intracellular accumulation of sorbitol or non enzymatic glycooxidation of proteins or both. The ascorbic acid concentration of leukocytes are significantly decreased in diabetes mellitus. Vitamin C could be important in preventing the glycooxidation of body protein in diabetes mellitus (Cunningham, 1998).

Vitamin C is a natural laxative and may help with constipation problems. Moreover, vitamin C provides a protection for cancer. Intake of vitamin C at moderate doses is effective in lowering the risk of developing cancers of the breast, cervix, colon, rectum, esophagus, larynx, lung, mouth, prostate and stomach (Iqbal et al., 2004).

1.10.8 The Deficiency of Vitamin C

The first symptom of vitamin C deficiency is fatique. Fatiques are subtle, precedes other symptoms, occurs at plasma concentration below nearly 20μmol/L (Levine et al., 1999). Scurvy is the disease associated with vitamin C deficiency.

Clinical manifestation of scurvy are weakness, aching in bones, joints, muscles, hyperkeratosis around hair follicles, perifollicular hemorrhages, petechiae, muscle and joint hemorrhages, teeth that loosen and fall out, poor wound healing, anemia and fewer convulsions (Vilter, 1980).

Neurotic disturbance consisting of hypochondriasis, hysteria and depression followed by decreased psychomotor performances have been reported in ascorbic acid deficiency. Vitamin C deficiency is often associated with gingivitis (Iqbal et al., 2004).

1.10.9 The Toxicity of Vitamin C

Toxicity, normally does not occur, since vitamin C is water-soluble and is regularly excreted by the body (Iqbal et al., 2004).

The adverse effects of vitamin C are related to doses. Diarrhea or abdominal bloating and nausea can occur when several grams are taken at once (Bsoul et al., 2004). Oxalate, one of ascorbate metabolite, are found in urine, so, the risk of renal-oxalate stone formation may increase (Levine et al., 1996).

1.11 The Total Plasma Proteins in Blood

Blood plasma is the protein–rich fluid which suspends the cellular component of the whole blood. Plasma proteins consist of three major groups; albumin (60% of total plasma protein), globulins (36%), fibrinogen (4%). The blood plasma may contain 40000 different proteins. The normal range is approximately 6.0 to 8.3 gm/dL in blood (Motrescu el al., 2006). All the proteins of the body have a function. Plasma protein production can be controlled by diet. The certain aminoacids are very important in the production of new plasma protein - cysteine, leucine and glutamic acid (Madden et al., 1939).

The plasma protein concentration is affected not only by the balance between the rates of synthesis and breakdown, but also by the changes in the amount and distribution of body fluids and the protein depletion (Carpentier et al., 1982). The protein synthesis, breakdown and oxidation can be affected by the absorption of aminoacid by the gut (Bilsborough and Mann, 2006). The branched chain aminoacids which are present in whey protein is beneficial for the athletes. When branched chain aminoacids are taken during the aerobic exercise, the net rate of protein degradation decreases (Campbell et al., 2007). Leucine oxidation increases during exercise (Gaine et al., 2005). A research showed that net protein oxidation respond to dietary protein intake (Russel et al., 2002).

2 THE AIM AND SCOPE OF THE STUDY

The main objective of this study is to investigate the acute effects of whey protein and vitamin C combination on the hematological parameters, including the red and white blood cells of the male students. The vitamin C, the whey protein and the combination of the whey protein and the vitamin C were studied. The blood pH and the total plasma protein in blood were also examined.

3 MATERIALS AND METHOD

3.1 The Subjects (Individuals)

Forty healthy young male individuals (age = 23.47 ± 1.98 yrs, weight = 75.92 ± 10.47 kg, height = 178.42 ± 6.49 cm) volunteered to participate in this study. Volunteers were recruited from the university students and randomized into one of four groups. Treatment groups were administered in a double blind, randomized study testing.

None of the subjects had consumed any whey protein–containing nutritional supplements, alcohols, vitamins for at least two weeks prior to the beginning of the experimental period. Subjects did not take any nutritional supplements and did not participate any physical activities during the experimental period.

The study procedure, the potential risks and the benefits of the whey protein and vitamin C were explained to subjects. Before the experimentation, a written informed consent was obtained from all subjects. Then, all subjects completed the first questionnaire (including the age, the weight etc..). After the experimentation, the second questionnaire was completed to determine the acute side effects of the whey protein and vitamin C on subjects.

Table 3.1 The Descriptive Statistics of the Subjects

Groups	Age (years)	Weight (kg)	Height (cm)
1	22.50 ± 1.35	75.60 ± 8.98	176.50 ± 5.27
2	23.90 ± 1.66	79.00 ± 8.84	180.30 ± 7.33
3	22.60 ± 2.31	74.30 ± 12.92	180.60 ± 6.13
4	24.90 ± 1.66	74.80 ± 11.61	176.30 ± 6.71
Total	23.47 ± 1.98	75.92 ± 10.47	178.42 ± 6.49

Table 3.2 The Test of Homogeneity of the Variances in the Subjects

	Levene Statistics	df 1	df 2	Sig.
Age	.625	3	36	.603
Weight	1.057	3	36	.380
Height	.482	3	36	.697

*** p<0.05**

3.2 The Supplements

In this experiment, there are two supplements. These are the whey protein (with vanillin) (Merck ^{TM}) and vitamin C (Merck ^{TM}).

The supplements and the control material were placed in bar-coded vials. The relevant subject consumed the related supplements which is in the bar-coded vial. Water with vanillin was used as the control material (placebo). Vanillin does not contain any sugar.

3.3 The Experimentation and The Blood Sampling

Before the experimentation, the participants were randomly assigned in one of the four groups. The first group or control group (n=10) was treated only with water containing vanillin (200 mL water). The "n" refers to the number of the participants in each group. The second group (n=10) was treated with 30 g whey protein with water (200 mL water). The third group (n=10) was treated with 1000 mg Vitamin C dissolved in water (200 mL water). The fourth group (n=10) was treated with the combination of vitamin C (1000 mg) and whey protein (30 g) with water (200 mL water). These groups are summarized in Table 3.3

Table 3.3 The groups in the experiment

1. Group	The Control Group – (Placebo)
2. Group	Whey protein
3. Group	Vitamin C
4. Group	Whey protein and Vitamin C Combination

Figure 2 The samples of blood

Venous blood samples were collected before supplementation and after 75 minutes of the ingestion of the interested supplement. Blood was taken into a Vacutainer tube (Vacuette™, containing EDTA, K_3 EDTA, K3E) by puncture (Becton Dickinson™ Instrument, BD). These samples were protected from sunlight.

3.4 The Hematological Parameters

The hematological parameters were measured using a hemogram instrument (Cell – Dyn ® 1700). Hematological values include the red blood cell (RBC), the white blood cell (WBC), the lymphocytes (LYM), the precursors of white blood cell (MID), the granulocytes (GRAN), the hemoglobin (HGB), the hematocrit value

(HCT), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), the mean cell hemoglobin (MCH), the red blood cell volume distribution width (RDW) and the platelets (PLT).

3.5 The Determination of pH

After the hematological analysis, the pH of the samples were immediately checked by a pH meter (Hanna Instruments ^{TM}).

The blood samples were centrifuged at room temperature at 4000 x g for 15 minutes. Then, the plasma of samples was allocated to storage tubes. The plasma of samples were stored immediately at – 80 ^{o}C for future processing.

3.6 The Determination of Total Plasma Protein in Blood

Total plasma protein of the WCG and CG were detected by Biuret reagent. 2.4 g $CuSO_4.5H_2O$ (Riedel- de Haen^{TM}), 9.6 g Na-K tartarate (Carlo Erba Reagent^{TM}), 300 mL 5N NaOH (Merck^{TM}) were used for the preparation of Biuret reagent.

The stock BSA (Bovine Serum Albumin) solution was adjusted to a concentration of 100 mg/mL for the standard curve of BSA. Several concentrations of BSA from stock solution were prepared in the following manner:

- O.1 mL from stock solution + 0.9 mL dH_2O = 10 mg / mL
- 0.2 mL from stock solution + 0.8 mL dH_2O = 20 mg / mL
- 0.4 mL from stock solution + 0.6 mL dH_2O = 40 mg / mL
- 0.6 mL from stock solution + 0.4 mL dH_2O = 60 mg / mL
- 0.8 mL from stock solution + 0.2 mL dH_2O = 80 mg / mL
- 1 mL from stock solution + 0.0 mL dH_2O = 100 mg / mL

After preparing the BSA standards, 0.1 mL sample from each dilution was taken to test tubes and 3 mL Biuret reagent was added. All the tubes were thoroughly mixed and allowed a 30 minutes waiting period. The absorbance was determined by using a spectrophotometer at O.D. 540 nm (722 Grating Spectrophotometer). The absorbances at 540 nm were read against the blank (0,0 mg/mL). A linear standard curve for BSA was constructed using the absolute amounts of BSA plotted against spectrophotometric values.

Finally, the amount of BSA from each sample was determined. The same procedure was applied to plasma samples. The total plasma protein of samples were calculated with a standard curve of BSA.

Figure 3 The solutions of plasma + Biuret reagent

3.7 The Statistical Evaluation

The data were shown as means ± standart deviation and analyzed by the SPSS program. Paired t-test was used for comparison between the pre-treatment and the post-treatment in the groups. One-way ANOVA was used for comparison the differences between the groups. The significance level was set for $p< 0.05$, at the beginning of the study.

4 RESULTS AND DISCUSSION

In this experiment, no significant differences were observed between the groups for the age, the height and the weight of the subjects. In addition, no important differences were observed for the performance during the experimentation.

4.1 The Hematological Results of Control Group

Table 4.1 shows the changes of hematological value within CG. As seen from the Table 4.1, there is a statistically significant difference between the pre- and the post- treatment of WBC, MID (the precursor of the white blood cell), GRAN value, HCT and the blood pH. This result may be related to the period of starvation (the prolonged starvation). The prolonged starvation period is considered as a stress condition for the body. In post-treatment, a slight increase in the blood pH may depend on composition of blood including the waste of amino acids during the starvation period. Freitag et al. (2000) studied that the acute starvation and the subsequent refeeding affect lymphocytes subsets and their proliferation in the cats. Freitag's experiment showed that the decrease in the number of leukocytes and the percentage of CD4+ cells were related to the prolonged starvation period (Freitag et al., 2000). The results in table 4.1 confirm Freitag's conclusion.

Table 4.1 Paired t- test results of control group (CG) (placebo group)

Variable	Pre(mean±std.dev.)	Post(mean±std.dev.)	t	p
WBC(K/µL)	5,99 ± 1,27	5,45 ± 0,96	2,67	,026*
LYM (K/µL)	2,36 ± 0,84	2,01 ± 0,50	2,11	,064
MID (K/µL)	0,42 ± 0,11	0,36 ± 0,11	2,71	,024*
GRAN(K/µL)	3,21 ± 0,70	3,09 ± 0,64	1,96	,081
RBC(M/µL)	5,39 ± 0,37	5,25 ± 0,28	3,27	,010*
HGB(g/dL)	14,8 ± 1,42	14,53 ± 1,59	2,01	,075
HCT(%)	43,1 ± 3,85	42,08 ± 3,76	2,52	,033*
MCV(fL)	80,18 ± 7,57	80,32 ± 7,66	-,90	,392
MCH(pg)	27,54 ± 3,04	27,73 ± 3,03	-,75	,470
MCHC(g/dL)	34,34 ± 0,96	34,47 ± 0,82	-,41	,691
RDW(%)	14,05 ± 0,95	13,78 ± 0,95	1,45	,181
PLT(K/µL)	227,2 ± 45,68	207,8 ± 59,57	1,90	,090
pH	7,48 ± 0,03	7,513 ± 0,02	-3,40	,008*

***p < 0.05**

4.2 The Hematological Results of Whey Protein Group

Table 4.2 shows the changes of hematological value in WPG and it was observed that there were no significant differences between the pre-treatment and the post-treatment except the RBC and the HCT value.

The GRAN value in the post-treatment exhibited higher mean value than the granulocytes value in the pre-treatment in WPG, however, these differences were not statistically significant.

Rutherfurd-Markwick et al. (2005) showed that the whey protein and the whey protein-containing compound have immune stimulatory properties. Mice, fed with milk modified protein diet containing the whey protein for 4 weeks showed highly significant increase in blood cell phagocytosis response compared with the mice, fed only with the modified milk protein or the control group (Rutherfurd-Markwick et al., 2005).

Table 4.2 The paired t- test results of the whey protein group (WPG)

Variable	Pre(mean±std.dev.)	Post(mean±std.dev.)	t	p
WBC(K/µL)	6,56 ± 2,18	6,26 ± 2,05	,97	,358
LYM (K/µL)	2,59 ± 0,84	2,26 ± 0,73	1,96	,081
MID (K/µL)	0,45 ± 0,18	0,41 ± 0,14	1,50	,168
GRAN(K/µL)	3,54 ± 1,31	3,59 ± 1,50	- ,26	,798
RBC(M/µL)	5,25 ± 0,19	5,15 ± 0,18	2,59	,029*
HGB(g/dL)	15,15 ± 0,58	14,90 ± 0,57	1,79	,106
HCT(%)	43,92 ± 1,81	42,91 ± 1,45	2,96	,016*
MCV(fL)	83,62 ± 2,08	83,40 ± 1,98	1,98	,080
MCH(pg)	28,86 ± 0,84	28,97 ± 1,06	- ,91	,389
MCHC(g/dL)	34,51 ± 0,44	34,73 ± 0,64	-1,61	,141
RDW(%)	13,97 ± 0,75	13,7 ± 0,10	1,17	,272
PLT(K/µL)	222,1 ± 41,46	223 ± 43,58	- ,09	,931
pH	7,47 ± 0,09	7,50 ± 0,03	- ,78	,453

*$p < 0.05$

In this experiment, when compared with table 4.1 (the placebo group- CG) the whey protein acutely showed a positive effect on LYM and MID values. That is mostly related with the decrease of these values in the post-treatment of the CG. In contrast, there were no significant changes within WPG.

The mean GRAN value in the post-treatment of CG was decreased with the relative mean GRAN value in the pre-treatment of CG, the mean GRAN value in the post- treatment of WPG was slightly higher than the mean GRAN value in the pre-treatment of the WPG.

The RBC value was significantly decreased in the post-treatment. This decrease could be explained due to the bleeding during the experimentation. Also the HCT value was decreased in the post-treatment of the second group. The hematocrit values provide an information relative to the percentage of red blood cells in the whole blood (Schmaier and Petruzzelli, 2003). In other words, the decreased HCT value depends on decrease in the RBC value.

The hemoglobin value was unchanged within WPG. The constant hemoglobin and hematocrit value might be related to the nutritional supplementation

such as the proteins (Edozien and Switzer, 1997). But, acute supplementation with whey protein did not affect the hematocrit and the hemoglobin values. The MCV, MCH and the MCHC values were not changed by the supplementation with the whey protein. Moreover; RDW, PLT and the blood pH values were not also significantly affected by the whey protein supplementation.

Rankin et al. examined the effect of acute and chronic ingestion of the whey protein and the casein on the immunity in the athletes. The blood samples were collected via venipuncture with heparin before and after 120 minutes ingestion of the supplement (20 g of whey or casein). The T- cell subsets (CD3, CD4, CD8), the proliferation of lymphocytes were unaffected by the protein source (Rankin et al., 2006). In WPG, the LYM and the WBC value were not significantly affected by the supplementation of 30 g of whey protein.

A preliminary study by Rutherford–Markwick and Gill have examined the immunomodulating activity of protein concentrates derived from the bovine milk whey in mice. These investigators demonstrated that IMUCARE^{TM} whey protein, the cheese whey protein and the soybean protein affect immune system. After 8 weeks of dietary intake, mice fed with IMUCARE^{TM} whey protein exhibited significant increase in the number of the accessory (CD40+) blood leukocytes, whereas the mice fed with cheese exhibited a decline in number of the circulating helper T-subset lymphocytes (CD4). The soybean protein did not change the antibody response (Rutherford–Markwick and Gill, 2005).

Milk-derived proteins influence the immune cell in different means. The composition of the dietary protein and the amount of the ingredients can be different. The various process of the whey protein preparation might modulate immune system by different means. In other words, the amount of the lactoferrin, the

glycomacropeptide, the β-lactoglobulin and the alpha-lactoalbumin can vary in the whey protein supplements with respect to the origin of the whey protein (cow, sheep, goat…etc). The sequences of genes encoding whey protein were first reported in 1984 for the rat and the mouse. The whey protein is abundantly expressed in mammalian epithelial cells. The expression level of whey protein genes can differ in time. The induction and the expression of the whey protein gene depend on the hormones (prolactin, glucocorticoid and insulin) and the cell-cell and the cell-extracellular matrix interactions (Mercier and Vilotte, 1993). Variant conclusions in literature may result from various amounts of the ingredients of the whey protein.

4.3 The Hematological Results of The Vitamin C Group

Table 4.3 The paired t- test results of the vitamin C group (VCG)

Variable	Pre(mean±std.dev.)	Post(mean±std.dev.)	t	p
WBC(K/µL)	6,57 ± 1,41	6,60 ± 1,04	-,22	,833
LYM (K/µL)	2,41 ± 0,41	2,35 ± 0,55	,44	,671
MID (K/µL)	0,46 ± 0,11	0,38 ± 0,06	3,21	,011*
GRAN(K/µL)	3,73 ± 0,86	3,86 ± 0,78	-1,13	,282
RBC(M/µL)	5,19 ± 0,24	5,08 ± 0,23	1,06	,316
HGB(g/dL)	14,85 ± 0,79	14,85 ± 0,93	,00	1,00
HCT(%)	43,29 ± 2,56	43,19 ± 2,36	,24	,815
MCV(fL)	83,44 ± 3,36	83,43 ± 3,19	,05	,959
MCH(pg)	28,64 ± 1,05	28,68 ± 1,17	-,22	,832
MCHC(g/dL)	34,33 ± 0,67	34,37 ± 0,85	-,18	,861
RDW(%)	13,60 ± 0,61	13,70 ± 0,92	-,46	,656
PLT(K/µL)	262,3 ± 45,86	259,8 ± 37,56	,37	,717
pH	7,56 ± 0,06	7,57 ± 0,05	-1,00	,343

*** p < 0.05**

Table 4.3 indicates the effects of vitamin C supplementation on the hematological values. As seen from Table 4.3, vitamin C supplementation did not significantly affect the hematological values except the MID. The MID value is related to the precursors of the WBC and the blasts. The metabolism is able to utilize vitamin C in two hours and the intestinally absorbed vitamin C can circulate freely in plasma, leukocytes and red blood cell (Iqbal et al., 2004).

After the ingestion of the vitamin C (1000 mg); the WBC, the GRAN, the LYM values were remained as constant in one to two hours in the VCG. A study conducted in 1981 that when 1g (1000 mg) of the vitamin C was given intravenously to healthy individuals, after one hour, the neutrophil motility and the leukocyte transformation in the blood of the subjects were significantly increased (Iqbal et al., 2004). In VCG, vitamin C was orally introduced to subjects. The route of supplementation (oral or intravenous) with the vitamin C might effect the results of the third group. In blood, the concentration of the vitamin C provided by the oral intake differs from the concentration of vitamin C which is intravenously administered. This differences in concentration may influence the function and motility of the granulocytes.

Bergman et al. observed the incubation of the peripheral blood mononuclear cell (PBMC) with the vitamin C. A 0,8 mL of cell suspension was incubated with 0,2 mg/mL vitamin C for 60 minutes. This study indicated that the incubation of human PBMC with the vitamin C caused significant increase in the number of particles phagocytosized by each individual cell (Bergman et al., 2004).

The dosage and the absorption ratio of vitamin C or absorbed amount in blood may distinctly impact on the function and the transformation of the granulocytes. In VCG, the granulocytes value in the post-treatment was slightly higher than the granulocytes value in pre-treatment, but, these differences were not significant.

Recent research showed that the ascorbic acid can potentiate the mobilization of iron from inert tissue stores and facilitates the incorporation of iron into protoporphyrin. After 8 weeks of vitamin C treatment, the hematocrit value had significantly increased in a hemodialysis patient (Tarng et al., 2001).

As a result; supplementation with vitamin C did not acutely and significantly influence the hematocrit value and other hematological values.

4.4 The Hematological Results of The Combination of The Whey Protein and Vitamin C Group

Table 4.4 The paired t- test results of the combination of the whey protein and the vitamin C group (WCG)

Variable	Pre(mean±std.dev.)	Post(mean±std.dev.)	t	p
WBC(K/µL)	6,81 ± 1,07	6,56 ± 1,17	1,27	,235
LYM (K/µL)	2,98 ± 0,68	2,41 ± 0,65	5,25	,001*
MID (K/µL)	0,48 ± 0,10	0,42 ± 0,10	2,25	,051*
GRAN(K/µL)	3,33 ± 0,57	3,69 ± 0,84	-2,34	,044*
RBC(M/µL)	5,39 ± 0,21	5,40 ± 0,20	-,25	,810
HGB(g/dL)	15,39 ± 0,58	15,24 ± 0,49	1,37	,205
HCT(%)	44,26 ± 1,65	44,48 ± 1,39	-,75	,472
MCV(fL)	82,09 ± 2,44	82,39 ± 2,26	-1,57	,152
MCH(pg)	28,55 ± 0,94	28,40 ± 0,93	1,93	,086
MCHC(g/dL)	34,76 ± 0,31	34,45 ± 0,41	2,06	,070
RDW(%)	13,55 ± 0,65	13,93 ± 0,71	-2,21	,054*
PLT(K/µL)	230 ± 44,72	222,4 ± 43,03	2,61	,028*
pH	7,54 ± 0,04	7,58 ± 0,04	-1,89	,092

*** p < 0.05**

Table 4.4 indicated that the supplementation with the vitamin C and the whey protein combination affected the hematological value. As seen from the table 4.4, the LYM, MID, GRAN, RDW and the PLT values significantly changed within the groups. However, there were no significant differences between the other values. In WCG, the LYM, MID and the PLT value in the post-treatment statistically decreased. The combination of the whey protein and the vitamin C had showed no positive impact on the lymphocytes and the MID values. On the other hand, there was a statistical increase in the GRAN and the RDW values. The increased GRAN value may be due to the positive influence of the combination of 1000 mg vitamin C and 30 g whey protein on the granulocytes.

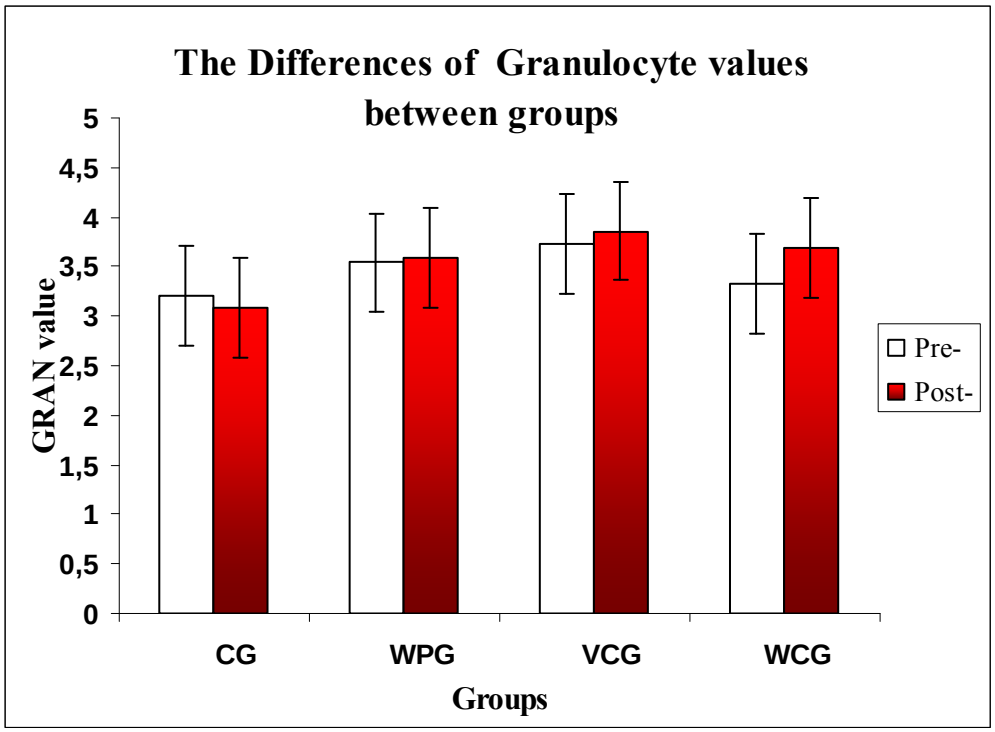


Figure 4 The differences of the granulocyte values between groups

Roth et al. studied the enhancement of neutrophil function by ultrafiltered bovine whey protein. The neutrophils were incubated in the dilutions of ultrafiltration bovine whey for 2 hours at 39 ^{o}C. In vitro treatment of bovine neutrophils with ultrafiltration bovine whey incubation statistically increased the random migration of neutrophils (Roth et al., 2001). Similarly, the researchers showed that vitamin C increased the transformation of leukocytes and help the monocyte–macrophage system (Iqbal et al., 2004). Some studies reported that the glycomacropeptides which is present in whey, increase the proliferation and the phagocytic activity of the macrophage like cell line (Daddaoua et al., 2005). Based on this information, acute supplementation of the combination of the whey and the vitamin C may stimulate the neutrophils or granulocytes in this study.

The hemoglobin concentration and the hematocrit and the MCHC values are very sensitive to the protein intake. Edozien et al. reported that the MCHC was

directly related to the dietary protein (Edozien et al., 1997). As seen from Table 4.4, there are no statistical significant differences between the pre- and post- treatment of of RBC, HGB, HCT, MCV, MCH and the MCHC value. These results may be related to level of the protein intake and the supplementation time. The RDW value in the pre-treatment was significantly different from the RDW value in the post-treatment. The RDW is calculated directly from the MCV. The RDW value shows the heterogeneity of the distribution of the red cell size (Schmaier and Petruzzeli, 2003; Park and Kim, 1987). The osmotic pressure in the blood and a change in the plasma volume could affect to the red blood cell membrane and the size. Thus the RDW value may be influenced by these events.

Soung et al. studied the soy protein supplementation. Women, fed by soy protein had an increase in the MCHC and the RDW value (Soung et al., 2006). The PLT value decreased in the post-treatment of the WCG. The decrease in PLT may relate to the bleeding during the experimentation.

Table 4.5 Anova

		Sum of Squares	df	Mean Square	F	Sig
WBC	**Between**	**1,641**	**3**	**,547**	**1,125**	**,352**
	Within	**17,510**	**36**	**,486**		
	Total	**19,151**	**39**			
LYM	Between	1,309	3	,436	2,021	,128
	Within	7,771	36	,216		
	Total	9,080	39			
MID	**Between**	**0.008**	**3**	**0.002**	**,421**	**,739**
	Within	**,228**	**36**	**0.006**		
	Total	**,236**	**39**			
GRAN	Between	1,193	3	,398	2,091	,119
	Within	6,846	36	,190		
	Total	8,039	39			
RBC	**Between**	**,132**	**3**	**0.004**	**1,139**	**,347**
	Within	**1,387**	**36**	**0.003**		
	Total	**1,519**	**39**			
HGB	Between	,457	3	,152	,837	,483
	Within	6,551	36	,182		
	Total	7,008	39			
HCT	**Between**	**12,069**	**3**	**4,023**	**2,988**	**,044**
	Within	**48,461**	**36**	**1,346**		
	Total	**60,530**	**39**			
MCV	Between	1,471	3	,490	1,788	,167
	Within	9,869	36	,274		
	Total	11,340	39			
MCH	**Between**	**,633**	**3**	**,211**	**,716**	**,549**
	Within	**10,607**	**36**	**,295**		
	Total	**11,240**	**39**			
MCHC	Between	1,614	3	,538	1,125	,352
	Within	17,210	36	,478		
	Total	18,824	39			
RDW	**Between**	**2,993**	**3**	**,998**	**2,424**	**,082**
	Within	**14,818**	**36**	**,412**		
	Total	**17,811**	**39**			
PLT	Between	2366,900	3	788,967	1,210	,320
	Within	23474,200	36	652,061		
	Total	25841,100	39			
pH	**Between**	**0.006**	**3**	**0.002**	**1,169**	**,335**
	Within	**0.006**	**36**	**0.001**		
	Total	**0.007**	**39**			

No statistical difference was observed between the groups (Table 4.5).

4.5 The Acid - Base Balance (The Blood pH)

The acid base homeostasis is achieved in part by the alternation of aminoacid metabolism. The aminoacid supplementation in the experimentation affect the acid – base balance, therefore the blood pH (Patience, 1990). On the contrary, in this experiment the combination of whey protein and vitamin C supplementation and the sole vitamin C or whey protein intake showed no significant changes in the blood pH.

4.6 The Results of The Total Plasma Protein of The Control and The Combination of The Whey Protein and The Vitamin C Group

Table 4.6 The Descriptive Statistics of Treatment and The Control Groups in The Total Protein Determination

	N	Age (years)	Weight (kilogram)	Height (cm)
The Treatment Group (Whey protein and Vitamin C)	10	24,90±1,66	74,80±11,61	176,30±6,72
The Control Group	10	22,50 ±1,35	75,60±8,98	176,50±5,28
Total	20	23,70±1,92	75,20±10,11	176,40±5,88

Table 4.7 The Paired T-test Results for Two Groups on the Total Plasma Proteins in Blood(mg/mL)

	Pre test	Post test	t	p
The Treatment Group	75,56±10,61	75,82±12,75	-,252	,80
The Control Group	89,12±5,42	84,89±4,64	2,275	,04*

*p<0.05

Table 4.8 The Independent Sample T-test Results for Total Plasma Proteins in the Blood(mg/mL)

The pre and the post test differences	Mean	df	Mean Differences	t	p
The Treatment Group **The Control Group**	-,26±3,26 4,23±5,87	18	-4,49	-2,112	,04*

*p<0.05

The turnover rate of the total plasma protein depends on the level of the dietary protein. Jeffay and Winzler showed that the half life of albumin was shortened and its replacement rate increased as the protein intake was raised. The hemoglobin level and hematocrit value remained unchanged in rats consumed various proteins for 3 days (Jeffay and Winzler, 1957; Carpentier et al., 1982).

The aminoacid concentration for the fast digesting aminoacid mixture and single whey protein meal rose rapidly at 20 minutes and lasted from 180–200 minutes. It was found that the fast digesting meals rapidly increased protein synthesis, did little to depress protein degradation and increased protein oxidation rates (Wilson and Wilson, 2006).

The metabolism of dietary proteins and the aminoacids are affected by the composition of the specific protein, the timing of ingestion, the composition of the meal and the dose of the protein. The speed of absorption by the gut of the aminoacids derived from dietary protein can modulate the whole body protein synthesis, the breakdown and the oxidation (Bilsborough and Mann, 2006). The concentration of the free aminoacids in the blood plasma are related to the level and aminoacids supplied by the diet (Forslund et al., 2000).

On the other hand, Bilsborough and Mann reported that the absorption rate of free aminoacids and the whey protein are greater than the egg white and the pea flour. The rapidly absorbed protein moderately inhibits the protein breakdown and

the stimulates protein synthesis. A single dose of 30 g of the whey protein or the free aminoacid, enhances the aminoacid oxidation (Bilsborough and Mann, 2006).

Pimenta et al. (2006) indicated that sedentary rats fed with the hydrolysate whey protein had high total proteins at the 48 h post-exhaustion. The hydrolysate whey protein promoted the biosynthesis of the serum albumin and the total protein.

The dynamics of serum protein response to acute changes in nutritional status. Serum transferrin and IgG decreased significantly with starvation and returned to baseline with repletion. Serum transferrin was the most responsive protein to acute, short-term nutritional depletion and repletion (Smale et al., 1980).

Consequently, in this experiment, there is a significant change in the control group. The total plasma protein in the post- treatment of the control group was lower than the total plasma protein of control group in the pre-treatment (

Table 4.7). The prolonged starvation time can be regarded as a stress condition for body. In the control group, the proteins may be sensitive to the oxidation due to the starvation. The antioxidant in the plasma may decreased during the prolonged starvation. Gönül and Kaplan (1999) reported that blood aminoacid levels decreased by starvation but increased by vitamin C supplementation. Vitamin C is a potent antioxidant. Plasma antioxidant sulfydryl group content in plasma also decreased with the unfed period.

Inayama et al. (2002) reported that the physical exercise induces oxidation of plasma protein thiols to cysteine-mixed disulfide in the humans. Moderate exercise induces the oxidation of the plasma protein. The total plasma protein is important for the physical exercise. In this experiment, no significant change in the total plasma proteins were seen with the whey protein and the vitamin C supplementation. The

vitamin C and the whey protein supplementation may stabilize the total plasma protein concentration. An acute supplementation of the whey protein and the vitamin C may affect the breakdown of protein and its oxidation.

5 CONCLUSION

It can be concluded that the acute consumption of the whey protein and the vitamin C, or both did not statistically affect the hematological value of the male. Ingestion of the combination of the whey protein and vitamin C may have a positive impact on the motility of the granulocytes and the GRAN value within fourth group when compared to the control group.

The decrease in the white blood cell and MID cell value may be related to the prolonged starvation period within the control or the placebo group when compared to the other groups. There is no change in the blood pH between the groups.

The 75 minutes time interval between the pre- and the post- treatment, the doses of the whey protein and the vitamin C, the change in the absorption of supplements in subjects can provide variations within the results. The vitamin C and the whey protein supplementation may stabilize the total plasma protein concentration, the protein oxidation and the protein breakdown.

In summary, hematological parameters were acutely effected by the ingestion of the combination of whey protein and vitamin C. The MCHC, MCV and the HCT values were not sensitive to acute protein intake. Therefore no side effects of the whey protein and the vitamin C supplementation was observed (stomach cramp, pain etc.).

6 REFERENCES

Aimutus, W.R., 2004. Bioactive properties of milk proteins with particular focus on anticarcinogenesis. Journal of Nutrition. 134: 989- 995.

Andrews, R.K., Lopez, J.A., Berndt, M.C., 1997. Molecular mechanism of platelet adhesion and activation. The International Journal of Biochemistry and Cell Biology. 29: 91 -105.

Andrijauskas, A., Iuaŝkevičius, J., 2006. New method of tracing blood hemoglobin concentration to hematocrit ratio for monitoring plasma dilution and osmotic region shifts in blood. Medicina (Kaunas). 42 (3): 181 -186.

Artym, J., 2006. Antitumor and chemopreventive activity of lactoferrin. Postepy higieny i medycyny doswiadczalnej. 60: 283 -352.

Audersirk, T., Audersirk, G., Byers, E. B., 2002. Biology Life on Earth. Prentice-Hall, Inc. USA. p. 621, 889.

Bergman, M., Salman, H., Djaldetti, M., Fish, L., Besler, I.P.H., 2004. In vitro immune response of human peripheral blood cells to vitamins C and E. Journal of Nutritional Biochemistry. 15: 45 -50.

Bilsborough, S., Mann, N., 2006. A review of issues of Dietary Protein Intake in Humans. International Journal of Sport Nutrition and Exercise Metabolism. 16: 129 -152.

Blomstrand, E., Eliasson, J., Karlsson, H.K.R., Köhnke, R., 2006. Branched- chain aminoacids activate key enzymes in protein synthesis after physical exercise. Journal of Nutrition. 136: 269 -273.

Blouet, C., Mariotti, F., Azzout-Marniche, D., Mathe, V., Mikogami, T., Tome, D., Huneau, J.F., 2007. Dietary cysteine alleviates sucrose – induced oxidative stress and insulin resistance. Free Radical Biology & Medicine. 42: 1089 - 1097.

Boririe, Y., Dangin, M., Gachon, P., Vasson, M.P., Maubois, J.L., Beaufrere, B., 1997. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proceedings of the National Academy of Sciences of the United States of America. 94: 14930 -14935.

Bozdoğan. Ö., 2004. Physiology. Palme, Ankara, Turkey. p. 524.

Bryant, C.M., McClements, D.J., 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat- denatured whey. Trends in Food Science & Technology. 9: 143 -151.

Bsoul, S.A., Terezhalmy, G.T., 2004. Vitamin C in health and disease. The Journal of Contemporary Dental Practice. 5 (2): 001 -013.

Campbell, B., Kreider, R. B., Ziegenfuss, T., Bounty, P.L., Roberts, M., Burke, D., Landis, J., Lopez, H. and Antonio, J., 2007. International Society of Sports Nutrition position stand: protein and exercise. Journal of the International Society of Sports Nutrition. 4: 1 -7

Carpenter, Y. A., Barthel, J., Bryns, J., 1982. Plasma protein concentration in nutritional assesment. Proceedings of the Nutrition Society. 41: 405 -417.

Cengiz, M., Cengiz, S., 2000. The effects of vitamin C administration on erythocytes glutathione and HbA1c level of type 2 diabetic patients. Cerrahpasa Journal of Medicine. 31 (4): 211 -215.

Colker, C.M., Swain, M.A., Fabrucini, B., Shi, O., Kalman, D.S., 2000. Effects of supplemental protein on body composition and muscular strength in healthy athletic male adults. Current Therapeutic Research. 61 (19): 19 -28.

Conneely, O.M., 2001. Antiinflammatory activities of lactoferrin. Journal of the American College of Nutrition. 20 (5): 389 -395.

Considine, T., Patel, H.A., Anema, S.G., Singh, H., Creamer, L.K., 2007. Interaction of milk protein during heat and high hydrostatic pressure treatment- A review. Innovative Food Science and Emerging Technologies. 8: 1 -23.

Cunningham, J.J., 1998. The glucose / insulin system and Vitamin C : Implications in insulin dependent diabetes mellitus. Journal of the American College of Nutrition. 17 (2): 105 -108.

Daddaoua, A., Puerta, V., Zarzuelo, A., Suarez, M.D., Sanchez de Mendina, F., Martinez-Augustin,O., 2005. Bovine glycomacropeptide is anti-inflammatory in rats with hapten-induced colitis. Journal of Nutrition. 135: 1164 -1170.

De WIT, J.N., 1998. Nutritional and functional characteristics of whey protein in food products. Journal of Dairy Science. 81: 597 -608.

Edozien J.C., Switzer, B.R., 1997. Effects of dietary protein and fat and energy on blood hemoglobin and hematocrit in the rat. Journal of Nutrition. 107: 1016 -1021.

Ferreira, I.M.P.L.V.O., Pinho, O., Mota, M.V., Taveres, P., Pereira, A., Gonçalves, M.P., Torres, D., Rocha, C., Teixeiraa, J.A., 2007. Preparation of ingredients containing an ACE inhibitory peptide by tryptic hydrolysis of whey protein concentrates. International of Dairy Journal. 17: 481 -487.

Formigomi, A., Piva, A., Pezzi, P., Castellani, G., Biagi, G., 2006. The influence of feeding fresh liquid whey on some blood metabolites, insulin and caecal fermentation of growing pigs. Animal Feed Science and Technology. 131: 52 - 65.

Forslund, A.E., Hambraeus, L., Beurden, H. V., Holmback, U., El-Khoury, A. E., Hjorth, G., Olsson, R., Stridsberg, M., Wide, L., Akerfeldt, T., Regan, M.., Young, v. R., 2000. Inverse relationship between protein intake and plasma free aminoacid in healthy mean at physical exercise. American Journal of Physiology – Endocrinology and Metabolism. 278: E857 -E867.

Freitag, K.A., Saker, K.E., Thomas, E., Kalnitsky, J., 2000. Acute starvation and subsequent refeeding affect lymphocytes subsets and proliferation in cats. Journal of Nutrition. 130: 2444- 2449.

Frid, A. H., Nilsson, M., Holst, J.J., Bjöck, I., 2005. Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetics subjects. American Journal of Clinical Nutrition. 82: 69- 75.

Fuenta, M.A.D.L., Hemar, Y., Tamehana, M., Munro, P.A., Singh, H., 2002. Process – induced changes in whey proteins during the manufacture of whey protein concentrates. International Dairy Journal. 12: 361 -369.

Gaine, P.C., Viesselman, C.T., Pikosky, M.A., Martin, W.F., Armstrong, L.E., Pescatello, L.S. and Rodriguez, N.R., 2005. Aerobic Exercise Training Decreases Leucine Oxidation at Rest in Healthy Adults. Journal of Nutrition. 135: 1088 -1092.

Gauthier, S.F., Pouliot, Y., Saint-Sauveur, D., 2006. Immunomodulatory peptide obtained by the enzymatic hydrolysis of whey protein. International Dairy Journal. 16: 1315 -1323.

Gönül, B., Kaplan, B., 1999. Effects of vitamin C supplementation on plasma antioxidant status in unfed periods. General Pharmacology. 32 (2): 195 –199.

Ha, E., Zemel, M.B., 2003. Functional properties of whey, whey components and essential aminoacid: mechanism underlying health benefits for active people (review). The Journal of Nutritional Biochemistry. 14 (5): 251- 258.

Hampl, J.S., Toylor, C.A., Johnston, C.S., 2004. Vitamin C Deficiency and Depletion in the United States: The Third National Health and Nutrition Examination Survey, 1988 to 1994. American Journal of Public Health. 94 (5): 870 -875.

Heddleson, R.A., Allen, J.C., 1997. Relative immunogenecity of β-lactoglobulin, bovine serum albumin, and a model acid whey product. Nutrition Research. 17 (3): 505 -514.

Inayama, T., Kashiba, M., Oka, J., Higuchi, M., Umegaki, K., Saito, M., Yamamoto, Y., Matsuda, M., 2002. Physical Exercise Induces Oxidation of Plasma Protein Thiols to Cysteine Mixed Disulfides in Humans. Journal of Health Science. 48 (5): 399- 403.

Iqbal, K., Khan A., Khattak, M.M.A.K., 2004. Biological Significance of Ascorbic acid (vitamin C) in human Health- A- Review. Pakistan Journal of Nutrition. 3 (1): 5 -13.

Jeffay, H., Winzler, R. J., 1957. The metabolism of serum proteins. The effect of dietary protein on he turnover of rat serum protein. The Journal of Biological Chemistry. 111- 116.

Kelleher, S.L., Dereck, C., Nielsan, K., Lönnerdal, B., 2003. Glycomacropeptide and α-lactalbumin supplementation of infant formula affects growth and nutritional status in infant rhesus monkeys. American Journal of Clinical Nutrition. 77: 1253 -1261.

Kırıkoğlu, O., 2002. M.S. Thesis. Abant İzzet Baysal University. Education of Training, Ankara, Turkey. p. 81.

Kontopidis, G., Holt, C., Sawyer, L.,2004. Invited Review: β- lactoglobulin: Binding properties, structure, and function. Journal of Dairy Science. 87 (4): 785 -96.

Kreider R. B., Earnest, C. P., Lundberg, J., Rasmusen, C., Greenwood M., Cowan, P., Almada, A. L., 2007. Effects of ingesting protein with various forms of carbonhydrate following resistance – exercise on substrate availability and markers of anabolism, catabolism and immunity. Journal of the International Society of Sports Nutrition. 4: 1 -18.

Levine, M., Cantilena, C.C., Wang, Y., Welch, R.W., Washko, P.W., Dhariwal, K.R., Park, J.B., Lazarev, A., Graumlinch, J.F., King, J., Cantelina, L.R., 1996. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. Proceedings of the National Academy of Sciences of the United States of America. 93: 3704 -3709.

Levine, M., Rumsey, S.C., Daruwala, R., Park, J.B., Wang, Y., 1999. Criteria and recommendations for vitamin C intake. Journal of the American Medical Association. 281 (15): 1415 -1423.

Madden, S. C., Finch, C. A., Swalbac, W. G., Whipple, G. H., 1939. Blood Plasma Protein Production and Utilization. The Journal of Experimental Medicine. 71 (3): 283 -297.

Marcus, A.J., Safier, l.b., 1993. Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis. Journal of the Federation of American Societies for Experimental Biology. 7: 516 -522.

Marshall, K., 2004. Therapeutic Application of Whey Protein. Alternative Medicine Review. 9 (2): 136- 156.

May, J.M., 1999. Is ascorbic acid an antioxidant for the plasma membrane? Journal of the Federation of American Societies for Experimental Biology. 13: 995 - 1006.

Mercier, A., Gauthier, S.F., Fliss, I., 2004. Immunomodulating effects of whey proteins and their enzymatic digest. International Dairy Journal. 14: 175 -183.

Mercier, J.C., Vilotte, J.L., 1993. Structure and function of milk protein genes. Journal of Dairy Science. 76: 3079 -3098.

Mikkelsan, T.L., Rasmussen, E., Olsen, A., Barkholt, V., Frokiaer, H., 2006. Immunogenecity of k-casein and glycomacropeptide. Journal of Dairy Science. 84: 824 -830.

Morifuji, M., Sakai, K., Sanbongi, C., Sugiura, K., 2005. Dietary whey protein downregulates fatty acid synthesis in the liver, but upregulates it in skeletal muscle of exercise- trained rats. Nutrition. 21 (10): 1052 -1058.

Morifuji, M., Sakai, K., Sugiura, K., 2005. Dietary whey protein modulates liver glycogen level and glycoregulatory enzyme activity in exercise- trained rats. Experimental Biology and Medicine. 230: 23 -30.

Motrescu, I., Oance, S., Rapa, A., Airine, A., 2006. Spectrophotometric analysis of the blood plasma for different mammals. Romanian Journal of Biophysics. 16 (3): 215 -220.

Nagatomi, R., 2006. The implication of alteration in leukocyte subset counts on immune function. Exercise Immunology Review. 12: 54 -71.

Paasch, S., Salzer, R., 2004. Solution to Spectroscopy challenge 7. Analytical and Bioanalytical Chemistry. 380: 734 -735.

Padayatty, S.J., Katz, A., Wang, Y., Eck, P., Lee, O.K.J.H., Chen, S., Corpe, C., Dutta, A., Dutta, S.K., Levine, M., 2003. Vitamin C as an antioxidant: Evalution of its role in Disease Prevention. Journal of the American College of Nutrition. 22 (1): 18 -35.

Padayatty, S. J., Levine, M., 2001. New insights into the physiology and pharmacology of vitamin C. Canadian Medical Association Journal. 164 (3): 353 -355.

Paddon-Jones, D., Sheffield-Moore, M., Katsanos, C.S., Zhang, W.J., Wolfe, R.R., 2006. Differential stimulation of muscle protein synthesis in elderly human following isocaloric ingestion of aminoacid or whey protein. Experimental Gerontology. 41 (2): 215 -219.

Park, K.I., Kim, K.Y., 1987. Clinical evaluation of red blood cell volume distribution width. Yonsei Medical Journal. 28 (4): 282 -290.

Patience, J.F., 1990. A review of the role of acid-base balance in aminoacid nutrition. Journal of Animal Science. 68: 398 -408.

Pimenta, F.M.V., Abecia-Soria, M.I., Auler, F., Amaya-Farfan, J., 2006. Physical performance of exercising young rats fed hydrolysed whey protein at a sub-optimal level. International Dairy Journal. 16: 984 -991.

Radak, Z., Chung, H.Y., Goto, S., 2008. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radical Biology & Medicine. 44 (2): 144 -153.

Rankin, J.W., Shute, M., Heffron, S.P., Saker, K.E., 2006. Energy restriction but not protein source affects antioxidant capacity in athletes. Free Radical Biology & Medicine. 41: 1001 -1009.

Ratner, A.J., Prince, A., 1999. Lactoperoxidase: New recognition of an "old" enzyme in airway defenses. American Journal of Respiratory Cell and Molecular Biology**.** 20: 642 -644.

Russell, K., Murgatroyd, P.R. and Batt, R.M., 2002. Net Protein Oxidation Is Adapted to Dietary Protein Intake in Domestic Cats. Journal of Nutrition. 132: 456 -460.

Rutherfurd-Markwick, K. J., Johnson, D., Cross, M.L., Gill, H.S., 2005. Modified milk powder supplemented with immunostimulating whey protein concentrate (IMUCARE) enhances immune function in mice. Nutrition Research. 25: 193 -203.

Rutherfurd-Markwick, K. J., Gill, H.S., 2005. Immunomodulatory activity of protein concentrates derived from bovine milk whey in mice. Nutrition Research. 25: 157 -166.

Roth, J.A., Frank, D. E., Weighner, P., Weighner, M., 2001. Enhancement of neutrophil function by ultrafiltration bovine whey. Journal of Dairy Science. 84: 824 -829.

Schmaier, A.H., Petruzzelli, L.M., 2003. Hematology for the medical students. Lippincott Williams & Wilkins. USA. p. 282.

Silvestro, A., Scopacasa, F., Oliva, G., Cristofaro, T.D., Luliano, L., Brevetti, G., 2002. Vitamin C prevents endothelial dysfunction induced by acute exercise in patient with intermittent claudication. Atherosclerosis. 165: 277 -283.

Smale, B.F., Mullen, J.L., Hobbs, C.L., Buzby, G.P., Rosato, E.F., 1980. Serum protein response to acute dietary manipulation. Journal of Surgical Research. 28(4): 379 – 388.

Smithers, G.M., Ballard, F.J., Copeland, A.D., De Sılva, K.J., Dioysius, D.A., Francis, G.L., Goddard, C., Grieve, P.A., Mcntosh, G.H., Mitchell, I.R., Pearce, R.J., Gegester, G.O., 1996. New opportunities from the isolation and utilization of whey proteins. Journal of Dairy Science. 79: 1454 -1459.

Soung, D.Y., Patade, A., Khalil, D.A., Lucas, E.A., Devareddy, L., Greaves, K.A., Arjmandi, B.H., 2006. Soy protein supplementation does not cause lymphocytopenia in postmenopausal women. Nutrition Journal. 5 (12): 1 -6.

Stevens, M.L., 1997. Fundamentals of Clinical Hematology. W.B. Saunders Company. USA. p. 393.

Tarng, D.C., Huang, T.P., Wei, Y.H., 2001. Erythropoietin and iron : the role of ascorbic acid. Nephrology Dialysis Transplantation. 16 (5): 35 -39.

Thompson, D., McNaughton, L., 2001. Antioxidant vitamins and muscle sorenes in humans: a brief review. Physical Therapy In Sport. 2: 141 -148.

Tipton, K.D., Elliott, T.A., Cree, M.G., Aarsland, A.A., Sanford, A.P., Wolfe, R.R., 2007. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. American Journal of Physiology-Endocrinology and Metabolism**.** 292: 71 -76.

Tkachuk, D.C., Hirschmann, J.V., McArthur, J.R., 2002. Atlas of clinical hematology. W.B. Saunders company. China. p. 154.

Tomita, M., Takase, M., Bellamy, W., Shimamura, S., 1994. A review: the active peptide of lactoferrin. Acta Paediatrica Japonica. 36 (5): 585 -91.

Vilter, R.W., 1980. Nutritional aspects of ascorbic acid: Uses and Abuses (Nutrition in Medicine). The Western Journal of Medicine. 133: 485 -492.

Wilson, J., Wilson, G.J., 2006. Contemporary Issues in Protein Requirements and Consumption for Resistance Trained Athletes. Journal of the International Society of Spports Nutrition. 3 (1): 7 – 27.

Wong, C.W., Seow, H.F., Husband, A.J., Regester, G.O., Watson, D.L., 1997. Effects of purified bovine whey factors on cellular immune functions in ruminants. Veterinary Immunology and Immunopathology. 56: 85 -96.

Zimecki, M., Artym, J., 2005. Therapeutic properties of proteins and peptides from colostrum and milk. Postepy higieny i medycyny doswiadczalnej. 59: 309 -23.

URL 1: http://upload.wikimedia.org/wikipedia/commons/8/81/Ascorbic_acid_structure .

7 APPENDIX

Appendix 1. The Hematological Data of the PRE –Treatment of the Control Group

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
3,6	1,4	0,2	2,0	5,55	14,5	44,1	79,4	26,1	32,9	14.0	220
8,1	4,5	0,6	3,0	5,76	16,2	47,7	82,8	28,1	34,0	14,0	251
6,6	2,5	0,5	3,6	5,07	14,4	41,5	81,9	28,4	34,7	13,0	192
6,4	2,1	0,4	3,9	5,00	15,2	44,0	88,1	30,4	34,5	13,8	327
7,1	2,3	0,5	4,3	5,70	17,0	48,6	85,2	29,8	35,0	14,5	255
5,9	2,0	0,3	3,6	4,79	14,3	40,0	83,6	29,9	35,8	12,9	229
5,2	2,2	0,4	2,7	5,59	14,5	41,5	74,3	25,9	34,9	14,2	172
5,1	2,2	0,4	2,5	5,84	11,7	35,8	61,3	20,0	32,7	16,1	187
6,7	2,7	0,5	3,5	5,52	15,7	46,2	83,7	28,4	34,0	14,8	193
5,2	1,7	0,4	3,0	5,11	14,5	41,6	81,5	28,4	34,9	13,2	246

Appendix 2. The Hematological Data of the POST–Treatment of the Control Group (CG)

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
3,5	1,1	0,2	2,2	5,36	14,6	42,6	79,4	27,2	34,3	14,2	161
6,0	2,9	0,4	2,7	5,54	16,3	46,0	83,1	29,4	35,4	13,7	226
5,7	1,8	0,4	3,5	5,16	14,5	42,9	83,1	28,1	33,8	13,1	208
6,1	1,9	0,3	3,9	4,96	15,3	43,9	88,6	30,8	34,9	13,5	323
6,7	2,2	0,5	4,0	5,52	16,7	46,9	85,0	30,3	35,6	14,1	267
5,0	1,6	0,2	3,2	4,70	13,3	39,2	83,3	28,3	33,9	12,9	239
5,1	2,2	0,4	2,5	5,49	14,2	41,0	74,6	25,9	34,6	14,3	176
4,6	1,8	0,3	2,5	5,48	11,0	33,5	61,1	20,1	32,8	15,1	159
6,5	2,5	0,5	3,6	5,20	14,9	43,2	83,2	28,7	34,5	13,8	117
5,3	2,1	0,4	2,8	5,09	14,5	41,6	81,8	28,5	34,9	14,0	202

Appendix 3. The Hematological Data of the PRE – Treatment of the Whey Protein Group (WPG)

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
7,3	1,9	0,5	4,9	5,46	15,2	44,0	80,5	27,8	34,5	15,2	185
6,2	2,2	0,5	3,5	5,27	15,5	45,2	85,7	29,4	34,3	14,4	162
7,0	2,7	0,5	3,9	5,05	15,1	42,7	84,5	29,9	35,4	12,6	172
6,6	2,6	0,4	3,6	4,99	14,3	41,1	82,3	28,7	34,8	14,0	273
5,1	2,1	0,4	2,7	5,15	15,4	44,3	86,0	29,9	34,8	14,2	232
12,1	4,7	0,9	6,4	5,46	16,2	47,4	86,9	29,7	34,2	14,0	261
5,5	3,0	0,3	2,2	5,38	15,5	44,7	83,1	28,8	34,7	13,3	249
5,3	2,1	0,4	2,9	5,18	14,8	42,9	82,9	28,6	34,5	13,8	273
4,0	1,8	0,2	2,0	5,07	14,3	42,0	82,8	28,2	34,0	13,4	207
6,5	2,8	0,4	3,3	5,51	15,2	44,9	81,5	27,6	33,9	14,8	207

Appendix 4. The Hematological Data of the POST – Treatment of the Whey Protein Group (WPG)

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
7,6	1,7	0,5	5,4	5,35	15,1	43,3	80,9	28,2	34,9	15,4	177
5,4	1,7	0,5	3,2	5,07	15,1	43,4	85,7	29,8	34,8	13,5	154
6,1	2,4	0,5	3,2	4,89	14,8	41,1	84,0	30,3	36,0	12,4	196
6,3	2,5	0,3	3,5	4,91	14,0	40,5	82,5	28,5	34,6	13,9	264
5,8	2,4	0,3	3,1	5,06	15,2	43,4	85,7	30,0	35,0	13,2	211
11,4	3,6	0,7	7,1	5,24	15,8	45,3	86,5	30,2	34,9	15,2	239
5,9	3,2	0,3	2,4	5,37	15,4	44,2	82,3	28,7	34,8	13,3	259
3,8	1,2	0,3	2,3	5,04	14,2	41,7	82,8	28,2	34,1	12,5	250
5,4	2,1	0,3	3,0	5,27	15,1	43,5	82,5	28,7	34,7	13,5	289
4,9	1,8	0,4	2,7	5,27	14,3	42,7	81,1	27,1	33,5	14,1	191

Appendix 5. The Hematological Data of the PRE – Treatment of the Vitamin C Group (VCG)

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
7,1	3,1	0,6	3,4	5,15	14,5	41,6	80,7	28,2	34,9	13,8	237
8,0	2,4	0,5	5,2	5,31	13,9	40,7	76,7	26,2	34,2	14,6	327
6,9	2,4	0,4	4,2	5,45	16,0	47,1	86,4	29,4	34,0	14,3	303
6,5	2,6	0,5	3,5	5,09	14,1	41,0	80,5	27,7	34,4	13,6	246
6,0	2,0	0,5	3,5	5,19	15,3	43,3	83,4	29,5	35,3	13,5	260
5,8	2,6	0,3	2,9	5,22	15,2	45,8	87,7	29,1	33,2	13,7	341
6,5	2,3	0,4	3,8	4,86	14,2	40,7	83,8	29,2	34,9	13,1	246
7,8	2,8	0,6	4,4	5,67	16,2	47,2	83,2	28,6	34,3	13,7	241
4,0	1,6	0,3	2,1	4,91	14,6	42,1	85,8	29,7	34,7	13,3	211
7,1	2,3	0,5	4,3	5,04	14,5	43,4	86,2	28,8	33,4	12,4	211

Appendix 6. The Hematological Data of the POST – Treatment of the Vitamin C Group (VCG)

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
7,3	3,5	0,4	3,4	5,23	14,4	42,7	81,6	27,5	33,7	13,9	253
8,3	2,4	0,4	5,5	5,24	13,9	40,4	77,1	26,5	34,4	15,4	320
6,7	2,5	0,4	3,9	5,27	15,5	45,4	86,1	29,4	34,1	13,2	273
6,9	2,3	0,4	4,2	5,38	15,0	43,3	80,4	27,9	34,6	13,1	246
6,5	2,4	0,5	3,5	5,29	15,6	44,0	83,2	29,5	35,5	13,4	283
5,5	1,6	0,3	3,6	5,02	15,2	44,2	88,1	30,3	34,4	13,6	301
6,6	2,4	0,3	3,8	4,88	14,1	40,6	83,2	28,9	34,7	13,1	265
7,4	2,8	0,4	4,2	5,74	16,7	48,0	83,6	29,1	34,8	15,1	246
4,5	1,8	0,3	2,4	4,85	14,4	41,0	84,6	29,7	35,1	13,8	218
6,3	1,8	0,4	4,1	4,9	13,7	42,3	86,4	28,0	32,4	12,4	193

Appendix 7. The Hematological Data of the PRE – Treatment of the Combination of Whey Protein and Vitamin C Group (WCG)

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
7,0	3,1	0,5	3,4	5,75	16,4	47,2	82,0	28,5	34,7	13,8	239
6,7	3,1	0,4	3,2	5,37	15,9	45,3	84,3	29,6	35,1	13,3	155
7,8	4,0	0,4	3,3	5,12	14,5	41,8	81,6	28,3	34,7	13,3	258
6,3	2,5	0,4	3,4	5,43	14,7	42,2	77,8	27,1	34,8	14,8	189
5,9	2,8	0,4	2,7	5,32	14,8	43,1	81,1	27,8	34,3	13,9	303
7,1	2,7	0,6	3,8	5,46	15,5	44,5	81,5	28,4	34,8	13,5	288
8,8	3,8	0,7	4,3	5,36	15,6	45,5	84,9	29,1	34,3	13,1	231
4,8	1,8	0,4	2,5	5,62	15,7	45,2	80,5	27,9	34,7	14,0	219
6,8	2,4	0,5	3,9	5,45	15,5	44,4	81,5	28,4	34,9	13,5	217
6,9	3,6	0,5	2,8	5,03	15,3	43,4	86,2	30,4	35,3	12,3	201

Appendix 8. The Hematological Data of the POST – Treatment of the Combination of Whey Protein and Vitamin C Group (WCG)

WBC (K/µL)	**LYM** (K/µL)	**MID** (K/µL)	**GRAN** (K/µL)	**RBC** (M/µL)	**HGB** (g/dL)	**HCT** (%)	**MCV** (fL)	**MCH** (pg)	**MCHC** (g/dL)	**RDW** (%)	**PLT** (K/µL)
6,3	2,5	0,4	3,4	5,67	15,9	46,7	82,3	28,0	34,0	14,3	234
6,5	3,0	0,3	3,2	5,25	15,6	44,2	84,1	29,7	35,3	13,7	163
6,9	2,9	0,4	3,6	5,18	14,6	42,4	81,8	28,2	34,4	14,0	259
7,0	2,6	0,4	3,9	5,50	14,8	43,3	78,8	26,9	34,2	14,2	186
5,2	2,0	0,3	2,8	5,31	14,7	43,1	81,1	27,7	34,1	14,6	284
7,9	2,0	0,4	5,4	5,61	15,8	46,0	82,0	28,2	34,3	14,8	289
8,0	3,1	0,6	4,3	5,42	15,9	45,6	84,2	29,3	34,9	12,9	217
4,1	1,1	0,4	2,6	5,41	15,1	44,0	81,3	27,9	34,3	14,6	202
6,8	1,9	0,4	4,4	5,59	15,8	45,4	81,2	28,3	34,8	13,4	204
6,9	3,0	0,6	3,3	5,06	15,1	44,1	87,1	29,8	34,2	12,8	186

Appendix 9. Blood pH of the Control Group (CG)

Pre-treatment		Post-treatment
7,52		7,52
7,46		7,51
7,46		7,51
7,54		7,54
7,45		7,53
7,47		7,51
7,46		7,51
7,48		7,48
7,47		7,52
7,50		7,51

Appendix 10. The Blood pH of the Whey Protein Group (WPG)

Pre-treatment		Post-treatment
7,50		7,49
7,46		7,50
7,48		7,44
7,49		7,47
7,48		7,51
7,48		7,54
7,52		7,55
7,51		7,49
7,51		7,50
7,49		7,51

Appendix 11. The Blood pH of the Vitamin C Group (VCG)

Pre-treatment		Post-treatment
7,46		7,50
7,55		7,57
7,47		7,51
7,53		7,55
7,55		7,61
7,67		7,68
7,56		7,55
7,63		7,55
7,57		7,58
7,67		7,73

Appendix 12. The Blood pH of the Combination of the Whey Protein and the Vitamin C Group (WCG)

Pre-treatment		Post-treatment
7,49		7,61
7,52		7,53
7,49		7,53
7,54		7,55
7,54		7,57
7,59		7,62
7,53		7,63
7,55		7,58
7,50		7,61
7,63		7,53

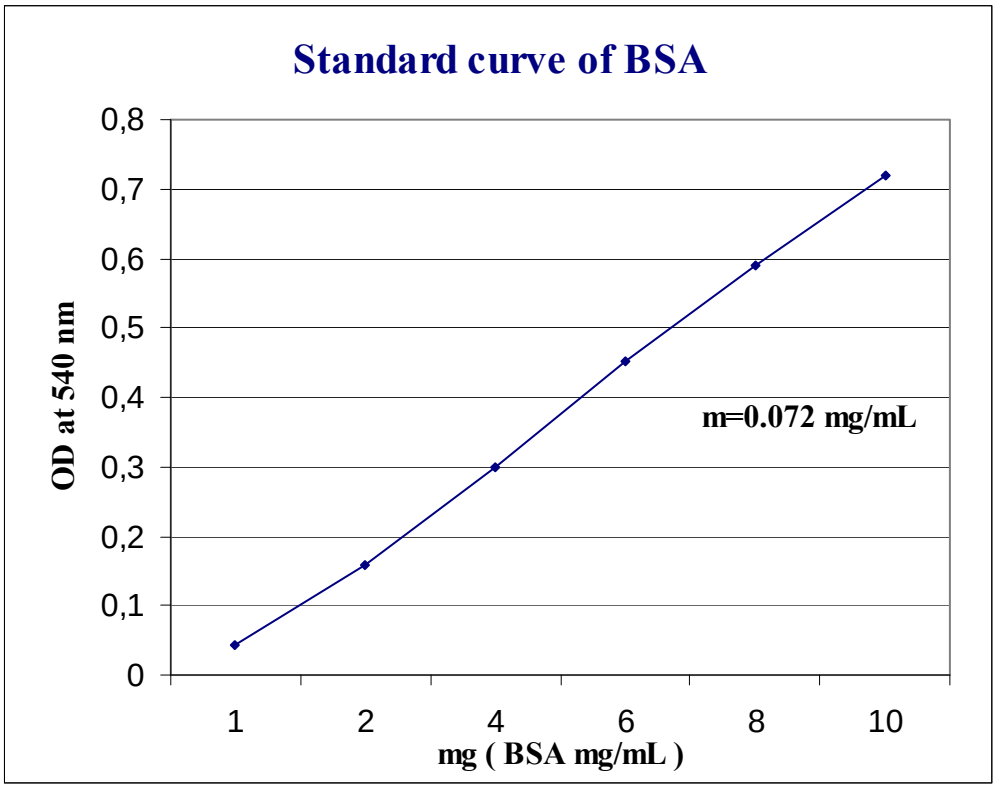
Appendix 13. The Results of Total Plasma Protein(mg/mL) in the Blood of the Control Group (sample size n = 10)

Pre-treatment O.D. $_{540}$	Post-treatment O.D. $_{540}$	Pre-treatment Protein Content (mg/mL)	Post-treatment Protein Content (mg/mL)
0,301	0,277	83,6	76,8
0,327	0,299	90,8	83,1
0,327	0,316	90,8	87,7
0.321	0,311	89,2	86,4
0,305	0,304	84,7	84,4
0,320	0,313	88,8	86,9
0,337	0,304	93,6	84,4
0,293	0,311	81,4	86,4
0,291	0,285	80,8	79,2
0,351	0,337	97,5	93,6

Appendix 14. The Results of Total Plasma Protein(mg/mL) in the Blood of the Combination of the Whey Protein and the Vitamin C Group.

Pre-treatment O.D. $_{540}$	Post-treatment O.D. $_{540}$	Pre-treatment Protein Content (mg/mL)	Post-treatment Protein Content (mg/mL)
0,252	0,248	70,0	68,9
0,274	0,264	76,1	73,3
0,285	0,283	79,2	78,6
0,174	0,163	48,3	45,3
0,277	0,284	76,9	78,9
0,287	0,279	79,6	77,5
0,288	0,282	80,0	78,3
0,271	0,277	75,3	76,9
0,313	0,340	86,9	94,4
0,300	0,310	83,3	86,1

Appendix 15. The Standard Curve of Bovine Serum Albumin



Appendix 16. The essential aminoacids which are present in the whey protein

Amino acid	Amount (mg)
Leucine	2937
Lysine	2436
Isoleucine	1418
Valine	1099
Threonine	960
Phenylalanine	884
Tryptophan	586
Methionine	471

Appendix 17. The non-essential aminoacids which are present in the whey protein

Aminoacid	Amount (mg)
Glutamic acid	3879
Aspartic acid	2359
Alanine	1161
Proline	950
Serine	899
Cysteine/Cystein	847
Tyrosine	790
Histidine	471
Glycine	434
Arginine	419