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A New Way to Treatment Various Diseases by Whey protein- A review

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ABSTRACT

Whey, a mixture of different proteins derived from milk and milk products, is now treated as a food with a number of beneficial health effects. Whey is a perfect example of how a unwanted by product is converted into a valuable raw material of many usage. It can be used in fermentation, production of whey protein concentrate (WPC) and whey protein isolate (WPI), lactic acid and bioethanol.It is also very famous because it's is a ready-to-drink beverage which offer the convenience and portability in our busy life.Whey proteins also is the preferred source of ready-to-drink protein beverages because of their excellent nutritional values, ease of digestibility (who are allergic to dairy products),bland flavor and have stronger shelf life which is important for a beverage.The biological components which includes lactoferrin, beta-lactoglobulin, alpha-lactaalbumin,glycomacropeptide and immunoglobulins contains a property of immune enhancing. Further, whey has properties like an antioxidant, antihypertensive, antitumor, hypolipidemic, antibacterial, antiviral and as a chelating agent.The whey proteins shows it's effect by intracellular conversion of cysteine(amino acid) to glutathione(potent antioxidant). Number of clinical trials have been successfully performed using the whey proteins, on several diseases.Aim of this project is to treat diseases like Cancer, Hepatitis, cardiovascular diseases, Obesity, Osteoporosis and HIV using whey products.

KEY WORDS: Whey, Whey protein concentrate(WPC), Immune enhancing, Antioxidant, Antibacterial.

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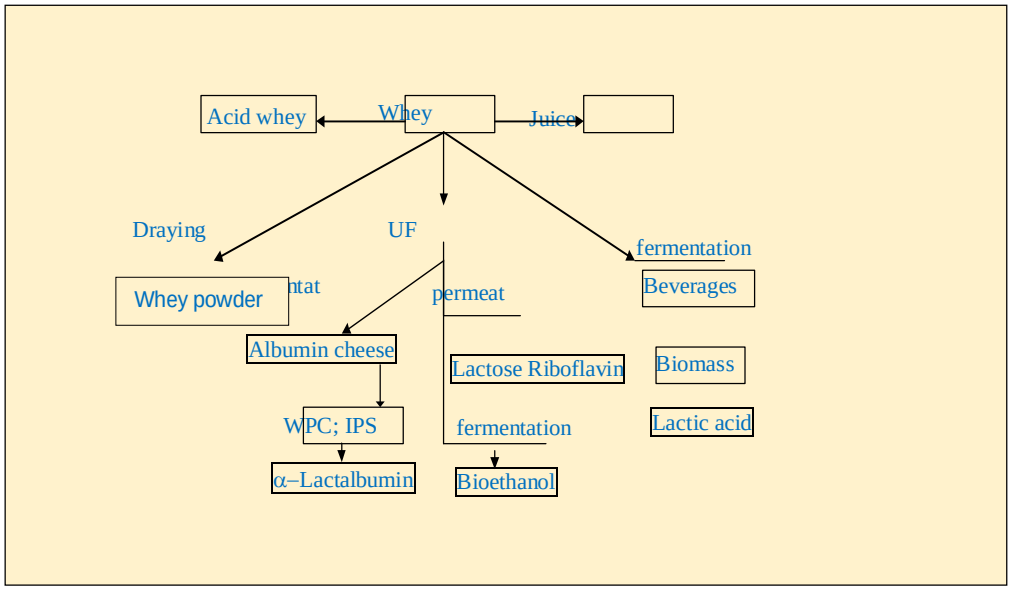
1.Introduction

In some of the recent years, constituents of milk have well been recognized in the category of functional foods, as their use has a direct and notable effect on human health.[1] Whey liquid, a by-product of cheese, curd manufacturing or casein production was being considered as a waste product. In 2008, total production of whey measured is 187 million metric tons out of which 3.2 million metric tons which is around 3.2% of the total volume was utilized by the several industries to manufacture whey powder, whey isolates, whey protein concentrates etc. In 2009 the sales of whey beverages increased about $1.03 million which is 6.3% increase than the previous year and it is growing rapidly than any other categories of beverages. [2] But it also have a adverse effect on our environment as it has a high biological oxygen demand (BOD)>35,000 ppm and the chemical oxygen demand(COD) > 60,000 ppm.[3] Milk contains two types of proteins which are casein one which is responsible for curdling of the milk and the other is whey which is a liquid phase protein source. This whey liquid contains beta-lactoglobulin, lactoferrin, lactoperoxidase enzymes, bovine serum albumin, glycomacropeptides, lactose, immunoglobulins (IgA, IgD, IgE, IgG, and IgM. IgG) and minerals.[4] Fermented foods are very well recognized as a part of a healthy diet in several different cultures all over the world. Previously whey was considered as a panacea (cure of all diseases) for problems ranging from joint and muscle to gastrointestinal. In Iceland the whey is called ‘syra’ which the Icelandic people used as a preservative for the raw meat and used in marinade in different food preparations. Whey also been fermented and stored in barrels to use it as a replacement of ale during the scarcity of grains in the region, described by a Icelandic food expert Nanna Rognvaldardottir[5]. Nowdays, whey is popular for their dietary protein supplement as well as to prevent diseases like cardiovascular incompatibilities and osteoporosis. The global increase of whey production has increased by 2% which symbolizes the parallel increase in milk production.[3] so, the researchers are trying to use whey in more efficient amount which will be economical too with contemporary manufacture of products like whey powder, whey protein isolate etc. This is important so that this kind of highly perishable things can be used properly.

Fig: 1



Possibilities of whey utilisation

Fig:2

Whey	Total solids	Lactose	Proteins	Calcium	Phosphates	Lactate	Chlorides
Sweet	63.0 – 70.0	46.0 – 52.0	6.0 – 10.0	0.4 – 0.6	1.0 – 3.0	2.0	1.1
Acid	63.0 – 70.0	44.0 – 46.0	6.0 – 8.0	6.0 – 8.0	2.0 – 4.5	6.4	1.1

Composition (g/L) of sweet and acid whey

2.Whey protein Manufacturing

Percentage of whey protein present in bovine whole milk is about 20% of the total protein. But, if the casein is removed by curdling then the remaining whey liquid contains 65% protein.The manufacturing method from Ohio State University of whey protein powder is given below.The milk is heated at high temperature and pasteurized (30 secs at 163 degrees F) then held at 40 degrees C for overnight. The next morning the mixture is cooled to the 30 degrees C, inoculated with a lactic acid culture and then incubated for the next 30 minutes. The mixture is stirred with adding Rennet Extract (Derived from stomach of newly born calves) and that leads to coagulation of curd. Chymosin, an active enzyme present in the rennet mixture is responsible for coagulation of the milk by disparate into curd and whey liquid. The enzyme chymosin helps in digestion and absorption of milk in newly born calves.

The liquid phased mixture is passed through a screen and the curd is separated. The whey liquid is filtered at 45 degrees C and the pH is adjusted to 3 by adding citric acid. The liquid is filtered to one-fifth to its original volume resulting in a whey concentrate containing 80% protein which can be further filtered using a micro filter increasing the protein concentration to 95% with fat and lactose.[6] The commercial success of whey protein as a supplementary food option has a influence of manufacturing it not only as a by-product of cheese and curd. Manufacturers now preserve whey with special care to maintain the biological activity as well as the protein structure and the protein bound fats. Whey is processed under low temperature and not exposed under fluctuating pH in terms of avoiding the denaturation of protein molecules.Also the health of the milking cows are thought to have a contribution in the immune-enhancing property of the whey based products.[7][8]

3. Biological Components Amino Acid Component

Whey proteins have all the essential amino acids and in concentrations comparatively higher than present in vegetable resources such as soybean, wheat,corn and gluten.[4] Whey has a high concentration of branched chain amino acids like valine, leucine, isoleucine etc. These amino acids, specially leucine is an important factor for repair and growth of tissue. Also, leucine leucine is recognized as a key amino acid of protein metabolism for the translation-initiation pathway of protein synthesis.[9]

Whey proteins also contains amino acids like cysteine and methionine. These amino acids are well known as immune enhancement by intercellular conversion to glutathione in high concentration.

3.1.Lactoferrin

Lactoferrin is a non-enzymatic antioxidant found in the whey liquid of milk. It's also found in colostrum. The human lactoferrin contains 691 amino acids where the whey contained lactoferrin have 689.[10] Lactoferrin is a iron binding glycoprotein with two binding sites for ferric ions. The lactoferrin which contains less than 5% iron (iron-depleted lactoferrin)is referred as apolactoferrin.Human breast milk contains apolactoferrin.[11] In bovine milks and colostrum contain lactoferrin 0.2mg/ml and 1.5mg/ml respectively.[12] Bovine lactoferrin is only 15-20% saturated with iron. In human milk and colostrum the concentration is approximately 2mg/ml and 7 mg/ml. It is an important component in human milk, but most commercial whey protein powder it contains in 0.35-20% concentration.

3.2. Immunoglobulins

An immunoglobulin is an antibody present in human body. It can be classified into five subtypes such as- IgA, IgD, IgM, IgG And IgE. IgG is found as the largest amount of antibodies in an adult (approximately 75%) .IgG is transformed from mother's body to child's via placenta and by breast milk.It is referred as "passive immunity" as it helps in the child's immunity. It has been seen by study that infant provided breast milk have better immunity than the bottle feed one's because of the IgA present in the breast milk.[13]. Colostrums contains more amount of immunoglobulin than present in the mature milk.

The whey phase of milk contains 10-15% immunoglobulins of the total proteins present in it. It has been inferred in a In-Vitro study of a bovine-milk that, IgG present in it the human lumphocyte proliferative response to T cells low as 0.3mg/ml of IgG. It is further concluded that the bovine milk IgG ranges in between 0.6-0.9 mg/ml. It is the amount that could be carried to human body.[14] Studies on milk also reveals that raw-milk from non-immunized cows can contain specific antibodies such as Salmonella enteridities , E. coli, Shigella flexneri, S.typhimurium.[14,15]

3.3. α –Lactalbumin

It is one of the main protein can be found in human and bovine milk. Approximately20-25% of whey proteins comprises of it and this contain a wide variety of amino acids including essential and branched chain amino acids. It is a good source of readily available supply of amino acids and so is used in infant food and drinks. But due to cost effectiveness,most dairy based infant food contains ingredients like demineralized whey with higher amount of β –Lactoglobulin which make them similar to human milk. In a study, it have shown that α-Lactoglobulin in both native and hydrolyzed state enhances the antibody stimulation against any antigen.[16] The same group of scientists also proved that this protein has a direct effect on T cell-dependent and independent responses and B -lymphocyte function.[17]

3.4. β-Lactoglobulin

This is the approximately present in half amount of the total protein present in the bovine milk whey. On the other hand human milk doesn't contain beta-lactoglobulin. As well as being a source of essential and branched chain amino acids, researchers have found s retinol-binding protein in it. β-Lactoglobulin is a carrier of hydrophobic molecules like retinoic acid which is known for the modulation lymphatic responses.[18]

3.5. Lactoperoxidase

Lactoperoxidase is an important enzyme which can be found in whey fraction. Whey contains various type of enzymes that includes lyases,proteases, Lipase, Transferases and hydrolyses. Lactoperoxidase is found in most abundant quantity and majority portion of it ends up in the whey following the curdling process. It constitutes 0.2-0.5% of total proteins found in whey. This enzyme can catalyze certain molecules like reduction of hydrogen peroxide.[19]This actually catalyzes peroxidation reaction of halides and thiocyanate which generate some product which can inhibit/kill some bacterial species.[20] Although during pasteurization, Lactoperoxidase is not inactivated since it have a stability as a preservative.

Fig:3

Whey Components	% of Whey Protein	Benefits
beta-Lactoglobulin	50-55%	Source of essential and branched chain amino acids
alpha-Lactalbumin	20-25%	Primary protein found in human breast milk Source of essential and branched chain amino acids
Immunoglobulins	10-15%	Primary protein found in colostrum Immune modulating benefits
Lactoferrin	1-2%	Antioxidant Antibacterial, antiviral, and antifungal Promotes growth of beneficial bacteria Naturally occurs in breast milk, tears, saliva, bile, blood, and mucus
Lactoperoxidase	0.50%	Inhibits growth of bacteria
Bovine Serum Albumin	5-10%	Source of essential amino acids Large protein
Glycomacropeptide	10-15%	Source of branched chain amino acids Lacks the aromatic amino acids phenylalanine, tryptophan,

Components found in whey protein

3.6. Glycomacropeptide

Glycomacropeptide also known as GMP is a casein macropeptide. It is present in whey at a concentration of 10-15% and is produced due to the action of chymosin on casein during the manufacturing of cheese. GMP can only be present in whey when chymosin is used during the cheese making process, however during the manufacture of some cheese chymosin is not used(cottage cheese)so GMP is not present in them.[21] GMP have high amount of branched chain amino acids and also it lacks of aromatic amino acids such as phenylalanine ,tryptophan, tyrosine. Due to lack of phenylalanine,it is suitable for patients have phenylketonuria(PKU).

3.7. Bovine Serum Albumin

Bovine serum albumin (BSA) is a protein can be found on whey 10-15% on concentrate of total protein in it. Though BSA is source of some essential amino acid but we have very little information about this protein and it's therapeutic effectiveness.

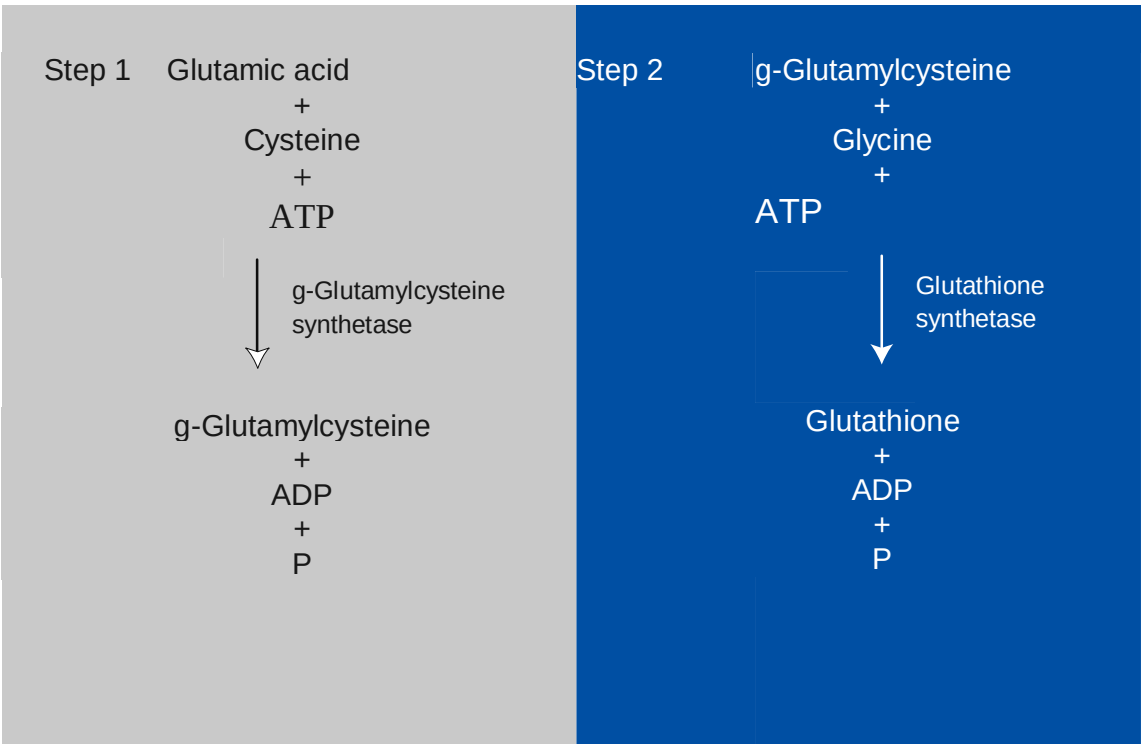
3.8. Mechanism Of Action

Whey shows potent antioxidant activity due to the presence of cysteine rich protiens which helps in the synthesis of glutathione(GSH) which is a potent antioxidant.[2] Cysteine contains a sulphydryl or thiol group that is an active reducing agent in preventing oxidation as well as tissue damage. Medical practitioners often use whey products as a good source of cysteine in order to increase the level of glutathione.[22,23] several studies on lactoferrin have showed it's effectiveness to activate the natural killer cells (NK) and neutrophils, enhanced macrophage toxicity and induced colony-stimulating factor activity.[24-27] .Lactoferrin also have several different properties such as antifungal, antibacterial and antiviral. The antimicrobial property of lactoferrin is more potent as compare to the other ones, it act's by making a chealting complex with iron that deprives microorganisms of this essential nutrient for growth.[28] Also lactoferrin has the ability to abolish the outer cell membrane of a gram(-) bacteria which is the lipopolysaccharide component, acting as a antibiotic.[29] In a another study on mouse it has been shown that it can reduce the inflammation due to it's ability to regulate the levels of interleukin 6(IL-6) and tumoe necrosis factor (TNF).[30]

Another component of whey is α-Lactalbumin which also have the ability to chelate the heavy metals.[31]It reduces the oxidative stress because of it's iron-binding properties.[32]

The β-lactalbumin present in whey give it the significant angiotensin I converting enzyme (ACE) inhibitory activity which prevents the production of angiotensin II from angiotensin I, angiotensin II is a potent vasoconstrictor. Therefore, whey recently is used as a healthy dietary supplement to reduce the blood pressure.[33,34]

Fig:4



Synthesis of glutathione from Cysteine, Glutamate and Glycine

4. Clinical Indications For Whey

4.1.Cancer

Whey protein recently have been studied extensively for it's effect in prevention and treatment of cancer. In a review of whey protein concentrates in the treatment of cancer, Bounous discussed it's antitumor and anticarcinogenic potential. The amino acis precursors to glutathione available in whey might:1) stimulate immunity 2) detoxification of potential carcinogens and 3) increase glutathione concentration on relevant tissues.[35]Others authors included that the iron binding ability also may contribute to it's anticancer property as iron can damage the tissues by oxidation. Many animal studies have been done to examine the effects of whey and as well as it's immune enhancing components like lactoferrin and β-lactalbumin. In animal study of colon cancer whey demonstrated significantly lower incident of tumors as well as low aberrant cyrpts.[36-43] Yoo et al stated lactoferrin has the ability to inhibit the metastasis of primary tumor cancer cells in mice. Bovine serum albumin (10-15%) acclaimed the inhibition of breast cancer cells in human(in

vitro).[42]In a recent clinal trial conducted with 20 patients persisting on stage IV malignancies.Bladder(1),Breast(5),prostate(2), neuroblastoma9(1),lymphoma(2),non small cell-lung(2) and osteocarcoma(1) were given a combination of denatured whey protein concentrate 40 g/day ,1-2 g/day oral ascorbic acid, 50-100g/day intravenous Transfer Factor Plus(it is a supplement containing number of immunoactive components) and Agaricus blazei tea, a multi-vitamin complex, a soy extract and 500 mg Andrographis paniculata twice a day and continued for 6 months.[44] After the time there were 16 survivors, all of them have higher mean haemoglobin and hematocrit levels and enhanced NK function. All patients showed improved quality of life during the study.

4.2.Antimicrobial

Plasma level of lactoferrin have found to be increased due to the release from neutrophils during any kind of infection, tumor development, inflammation.[12] several studies have explained that lactoferrin plays a dominant role in the body's defense mechanism. In addition people founded with lower level of lactoferrin have higher risk of infection than others with normal concentration.[45,46] In a single center study of 150 individuals infected with H.pylori were given antibiotics at different doses and durations (7-10 days) with 200 mg encapsulated lactoferrin.[47]Analysis of the study revealed 100% eradication of the infection of the group trated with seven days antibiotic course with addition of lactoferrin. On the other group the success is 76.9% treated with only antibiotic course. The same study in 10-day treatment group showed only 70.8% success rate.

4.3.Hepatitis

Whey protein supplementation demonstrate different effects in patients infected with Hepatitis B or C. Initially it was found that bovine lactoferrin prevented hepatitis C virus (HCV)in vitro in a human hepatocyte line.[[48] A random study was conducted in 11 patients with chronic HCV. Each patient given 1.8 or 3.6 g bovine lactoferrin daily for 8 weeks. In patients who have low pretreatment viral loads of HCV RNA and serum alanine transaminase were observed. But, in patients with higher pretreatment HCV viral loads, levels were did not changed significantly.[49]In an other study conducted on 25 patients with either infected with Hepatitis B or C , patients were given 12 g whey(Immunocal) twice daily for 2 weeks. Patients also given casein following Immunocal for a four-week duration. In the 17 patients with HCV , no significant changes had been noted. But in the group patients infected with hepatitis B virus(HBV) IL-2 and NK activity enhanced. Serum lipid peroxidase levels decreased. In 6 out of 8 patients serum alanine transferase levels were reduced and plasma glutathione levels increased in 5 out of the 8. The clinical trial indicates for the use of the use of whey protein in the treatment of HBV.

4.4. Human Immunodeficiency virus(HIV)

Glutathione deficiency is a very common problem in HIV patients. As we know it synthesizes from cysteine; so, to increase the cysteine level which leads to the elevated level of glutathione several studies have been conducted in HIV positive patients.[50,51] In a stusy in Micke et al, 30 HIV patients were randomly given a dose of 45g whey protein from one of the two sources- 1.Protectamin® 2. Immunocal. The two products have different amino acid concentration and immunocal is produced in a lower isolation temperature which is <72◦ C. After 2 weeks analysis have shown that the group provided Protectamin have increased levels of glutathione. On the other hand the other group who's were provided Immunocal had no significant elevation of glutathione level.

The same group of researchers conducted a study on the same topic on a longer duration of 6 months using the same dose and products.[51] As expected the protectamin group showed increased level of glutathione within two weeks period while the immunocal group did not. All subjects were then crossed over to receive protectamin. After six months all patients have significantly increased glutathione level as compared to the initial values.

4.5.Cardiovascular Disease

The clinal studies have shown that cardiovascular diseases(CVD) have a linkage with high fat diet intake. Though CVD is linked to a couple of other factors like age, genetics, obesity, lifestyle,

alcohol intake and the quality of the fat taken by everyday diet. Milk contains more than 12 different distinctive types of fat includes free sterols, cholesterol, sphingolipids and oleic acid.[52] Several studies have found that milk and the milk products lower the risk of hypertension.[53-55]

A study was conducted on 20 individuals (adult male) to investigate whether a fermented milk supplement with addition of whey protein concentrate would affect their blood pressure and serum lipid levels.[56] During the course of 8 weeks volunteers divided into two group. One group consumed 200 mL of fermented milk with whey protein concentrate and the other one given placebo. The placebo contains a non-fermented milk product without the addition of whey protein concentrate. After 8 weeks the first group showed significant elevated level of HDLs with lower triglycerides and systolic blood pressure. While the other group's total cholesterol and LDL levels were lower.

4.6. Obesity

Obesity has reached epidemic proportions in the world and specially countries like United States. Low fat, high carbohydrate dietary trends are being evolved for higher protein and non carbohydrate diets. Whey is becoming an attractive source of dietary protein. Whey protein isolate's protein concentration can be as high as 95% after removal of fat and lactose. Whey has a significant value in weight loss industry. The non essential and essential amino acids present in whey act as precursor for protein synthesis and can improve body mass index in individuals with exercise. Zemel et al explained a greater effect of diary versus non diary souces of calcium for improving the body composition. A study was held in mice with diet of calcium-fortified cereal with calcium-fortified cereal + nonfat dried milk to show the accelerating weight and fat loss. The mice given the nonfat died milk showed greater amplification of mass and fat loss. Although the mechanism responsible for such result is still unknown. [57] The bioactive components present in whey are thought to act additively with calcium to attenuation of the lipogenesis process, accelerated lipolysis and effect in nutrient portioning between adipose tissue and skeletal muscle.[57]

4.7.Osteoporosis

Milk is well known and is widely proposed as a nutritional food that helps in osteoporosis due to its calcium content.[58] Researchers have been trying to figure out the main constituent of milk responsible for such action. Initially, based on in-vitro and animal studies milk basic protein(MBP) is considered as the component with ability to stimulate proliferation and differentiation of osteoblastic cells as well as suppression of bone resorption.[59-61] MBP can be prepared from fractionized whey through cation exchange resin. Several in-vivo studies on mice have sorted out that both the whey protein and fractionated whey protein has the ability to increase femoral bone strength.[62-64]

In a clinical trial on 30 healthy adult men, tey were given 300 mg MBP.[65] The volunteers wer given the drink daily for 16 days with a normal diet. After 16 days, urinary calcium and serum calcium levels were changes from the baseline. In addition procollagen I carboxy-terminal propeptide(PICP) and serum osteocalcin levels increased which indicates increased bone formation. They are the biochemical markers for bone formation.

A another study conducted on 30 women over a 6 month duration. They were given either a placebo or a 40 mg MBP beverage daily. Results have shown the group given 40 mg MBP per day their radial bone density was increased significantly.[66]

4.8.Exercise

Whey protein supplements are very popular in body building world due to its high protein content. It has been speculated that a particular protein for enhancing muscle hypertrophy and strength is related to it's concentration of leucine.[32]

Burke et al explained that men who are engaged with resistance training program while supplemented of whey protein showed greater improvements in strength than compared to the people who only used the resistance training programs.[67] In a study 42 men, ages ranging between 18-31 and familiar with weight training were went through heavy-load and free-weight resistance for 12 weeks. They were divided into three different groups based on their diet. A gruoup of people given whey protein(1.2g/kg body wt/day) another group given a multi ingredient whey protein sports supplement (1.3 g/ Kg body wt/day) and the last groupis given placebo (12 g/Kg body wt/day). Intially there were no significant differences than baseline among the groups in lean tissue mass or strength. After 12 weeks group that received whey protein or whey protein sports supplement have showed better performance in resistance training . In addition they have shown better improvements in lean tissue mass than in place of placebo group.

5.Conclusion

As milk is considered as one of the oldest functional food .As whey liquid is also a fraction of milk and several profitable compounds for health can be found in it, so it is also can be as important source of proteins as milk is. Though, it whey was well known as a healthy compound from primitive age but the active compound were not well known before past couple of decades when different studies have done on this. To date, some adverse reaction have been showed up on administration of whey protein products, specifically to the dairy allergic people. It may not be suitable for them but some of category people have shown sensitiveness only to the casein part but they can tolerate whey phase of milk.

Hydrolyzed whey contains readily available di- and tri-peptide fractions which are nowdays very popular among athletes and other individuals due to it's quick absorbing power and low allergenicity protein source.The immune enhancing property of whey is now used by the medical practitioners to enhance the immune property in immune-compromised patients and can also be used as an antimicrobial agent.The recent studies have shown that anabolic response to essential amino acid with whey protein composition is greater than whey protein alone. Another study on influence of Ph and ionic strength on the physical and rheological properties and stability of whey protein stabilized o/w emulsion containing xanthan gum have studied recently. An recent study on aged rats have showed that "A meal with mixed soy/ whey proteins is as efficient as a whey meal in counteracting the age related muscle anabolic resistance only if the protein content and leucine levels are increased", but yet not studied in human body. Use of mass spectrometry to profile peptides in whey protein isolate medium fermented by Lactobacillus helveticus LH-2 and Lactobacillus acidophillus La-5 , also been studied recently. Whey protein edible films modified with almond or walnut oils through emulsification process is produced for food applications.

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