Canadian Science Publishing | Applied Physiology, Nutrition, and Metal

# Whey protein supplementation and its potentially adverse effects on health: a systematic review

| | |
|---|---|
| Journal: | *Applied Physiology, Nutrition, and Metabolism* |
| Manuscript ID | apnm-2020-0370.R2 |
| Manuscript Type: | Article |
| Date Submitted by the Author: | 24-Jun-2020 |
| Complete List of Authors: | Vasconcelos, Quezia; Universidade Federal do Ceará<br>Bachur, Tatiana; Universidade Estadual do Ceará Curso de Medicina<br>Aragao, Gislei; Universidade Estadual do Ceara, ; |
| Novelty bullets: points that summarize the key findings in the work: | • A systematic review of experimental and randomized studies about the use of whey proteins supplements and its impact on phys, • Analysis revealed that chronic and without professional guidance use of whey protein supplementation may cause some adverse, • Presented data support a need for future studies co-relating the use of different types of whey protein with and without exe |
| Keyword: | Hyperproteic diet, Whey protein, Hepatotoxicity, Adverse effects, Protein supplementation, Negative effects |
| Is the invited manuscript for consideration in a Special Issue? : | Not applicable (regular submission) |
| | |

# Whey protein supplementation and its potentially adverse effects on health: a systematic review

Quezia Damaris Jones Severino Vasconcelos[1]; Tatiana Paschoalette Rodrigues Bachur[2]; and Gislei Frota Aragão[1,2*]

[1]Faculty of Medicine, Drug Research and Development Center, Federal University of Ceara, Ceará, Brazil.

[2] Health Science Center, Ceará State University, Ceará, Brazil.

**Corresponding author:** Gislei Frota Aragao (gislei.frota@uece.br). Núcleo de Desenvolvimento de Medicamentos - NPDM/UFC, Rua Coronel Nunes de Melo, 100. Rodolfo Teófilo, Fortaleza – Ceará, Brazil. Telephone: +55 (85) 33668000.

**Contact information for other authors:**

1. Quezia Damaris Jones Severino Vasconcelos, queziaseverino@gmail.com
2. Tatiana Paschoalette Rodrigues Bachur, tatiana.bachur@uece.br,
3. Gislei Frota Aragao, gislei.frota@uece.br

**Abstract**

Whey protein is composed of soluble whey proteins and its benefits are well described in the literature. However, there are not many studies investigating the potential adverse effect of a diet with indiscriminate use of this supplement. The aim of this study was to perform a systematic review of papers that addressed this theme. A search was conducted in Medline, Lilacs, Toxnet, Web of science, and Scopus electronic databases. In the end, 11 documents composed this review. The majority of the papers associated the damaging effect with the chronic and abusive use of whey protein, the kidneys and liver being the main organs affected. The other studies related whey protein to aggravation of aggression, presence of acne and modification of the microbiota. Therefore, excessive consumption over a long period of protein supplementation may have some adverse effects on the body, being aggravated when associated with sedentary lifestyle. **PROSPERO registration number:** CRD42020140466.

**Novelty Bullets:**

- A systematic review of experimental and randomized studies about the use of whey proteins supplements and its impact on physical health.
- Analysis revealed that chronic and without professional guidance use of whey protein supplementation may cause some adverse effects specially on kidney and liver function.
- Presented data support a need for future studies co-relating the use of different types of whey protein with and without exercise to better see the impact on human physical health.

**Keywords**: Hyperproteic diet; Whey protein; Negative effects; Hepatotoxicity; Protein supplementation; Adverse effects.

## 1. Introduction

The two main sources of protein in milk are caseins and whey proteins, the casein being responsible for curdling, and the whey proteins referring to the aqueous part of the milk (Walzem et al. 2002). Today, after enzymatic processing it is possible to extract the proteins present in this serum including α-lactalbumin (50%), β-lactoglobulin (25%), bovine serum albumin (7%) and immunoglobulins (5%), which are rich in branched-chain amino acids (leucine - LEU, isoleucine, and valine) that thereby define and constitute the so popular Whey Protein (WP) (Aimutis 2004; Haraguchi et al. 2009).

Studies have shown that WP is the most widely used protein supplement among athletes and physical activity practitioners, since it plays an important role in protein synthesis, increasing lean muscle mass, and in the metabolism of carbohydrates that provide energy at the time of exercise, thereby improving sporting performance so highly (Borsheim et al. 2002; Yoshizawa 2004; Pasiakos et al. 2015).

It is estimated that up to 90% of all athletes worldwide consume dietary supplements at some level mainly because they are easily accessible (Huang et al. 2006; Denham 2011; Giannopoulou et al. 2013). In particular, amateur athletes often use WP based on their own prescription, usually obtained directly from sellers or through websites, ignoring the possible risks associated with chronic and excessive intake of a particular nutrient, such as proteins (Walter 2001; Allen and Haskell 2002; Laure and Binsinger 2005).

The benefits of using WP in clinical practice are very well described in the literature, such as effects on immunomodulation, antibacterial, antiviral, and antifungal activity among others (Vasconcelos et al. 2018). However, there are not many studies investigating the safety and potential adverse effects of a diet with indiscriminate use of these protein supplements (Wong et al. 2011; Lopez-Legarrea et al. 2014). Some studies have already shown that the exacerbated use of WP may be detrimental to health in the long term (Wakefield et al. 2011; Gurgen et al.

2014). Among the adverse effects are the increase in the presence of acne (Pontes et al. 2013), dysfunction of the microbiota (Moreno-Perez et al. 2018), and modifications in the regular metabolism of the kidneys (Aparicio et al. 2011) and liver (Gurgen et al. 2014). Thus, the objective of this study was to conduct a systematic review of the possible adverse effects caused by the indiscriminate use of WP and to discuss its potential harm to the body.

## 2. Materials and methods

The purpose of this systematic review was to find documents dealing with harmful and/or adverse and/or deleterious effects of WP supplementation on animals and/or humans. This study was registered at the International Prospective Register of Systematic Reviews (PROSPERO: CRD42020140466).

### 2.1 Search strategy and study selection

Five electronic databases were used: Medline (via Pubmed); Lilacs (via Portal Virtual Health Library - VHL); Toxnet (via Toxline); Web of Science, and Scopus (via Portal Periodical of Capes). The descriptors based on the Medical Subject Headings (MeSH) used in the search were: 'Whey Protein', 'Adverse Effects', 'Dietary Supplementation' and 'Toxicity', as well as their respective references in Health Science Descriptors (DeCS). The search terms were adapted for use with other bibliographic databases in combination with database-specific filters for controlled trials, where these are available.

The search strategies used were: 'Whey protein' AND 'Adverse Effects'; 'Whey protein' AND 'Toxicity'; 'Whey Protein' AND 'Dietary Supplementation'; 'Whey protein' AND 'Adverse Effects' AND 'Dietary Supplementation', as well as their respective variations in Portuguese. The period of publication of the articles selected was between 2000 and 2020, and the search carried out from September, 2018 to April, 2020. It was used articles in English and/or Portuguese. The full text of the potentially eligible studies was retrieved and independently assessed for eligibility by two review team members (QDJSV and TPRB). Any disagreement

between them over the eligibility of particular studies was resolved through discussion with a third reviewer (GFA).

**2.2 Data extraction**

The data collection was made manually by QDJSV. Each study quality was assessed by QDJSV and TPRB. The data were analysed after the division into subgroups: human and animal models, always comparing the same species with each other. The different results that the adverse effects of whey protein promoted were also analysed, such as increase expression of anger, exacerbation of acne, and damaging effect in gut microbiota, kidney, and liver.

**2.3 Eligibility Criteria**

Study inclusion was decided by a consensus between authors QDJSV and TPRB. In cases of disagreement with the inclusion of an article, GFA was consulted. Inclusion criteria were defined according to the PICOS approach (Liberati et al. 2009). Study selection criteria 1: Population: Humans (adults) and/or Animals; Intervention: whey protein or protein-based supplements; Comparator: control groups; Outcome: any adverse effect; Study design: randomised controlled trials, case-control (if relevant), and prospective. PICOS are described in table 1.

The exclusion criteria were: review articles, those that were not related to the intended subject, those that were out of the period of publication established, as well as those in languages other than the ones that were chosen. Regarding the inclusion criteria, we selected original articles that dealt with WP adverse effect in humans or experimentally in animals, published in Portuguese or in English between the years 2000 and 2020. A narrative synthesis of the findings from the included studies and the individual data from all randomized participants was evaluated, and all interpretation of the results from prospective randomized studies was performed on an intention-to-treat analysis.

### 2.4 Quality assessment

The research was conducted by three reviewers. Data supplied for included randomized control trials (RCTs) were checked for: missing data; internal data consistency; randomisation integrity (balance of patient characteristics at randomisation, pattern of randomisation); follow-up and censoring pattern. Summary tables were checked with trial protocol and latest trial report or publication. Any discrepancies or unusual patterns were checked with the study investigator. Final copies of the form from each trial were returned to the appropriate trial investigator for verification. Besides, articles were screened to identify if prospective registration of the clinical trial was conducted.

## 3. Results

### 3.1 Summary of findings



A total of 2345 articles were retrieved from the initial research, and only 1656 remained after duplicates removal. After reading titles and then abstracts, two review authors (QDJSV and TPRB) identified studies that potentially meet the inclusion criteria remaining only 23 relevant papers. Based on the exclusion criteria, 12 articles were excluded and an additional four papers found by other sources were added. The primary reasons for exclusions are listed in figure 1, some papers had multiple reasons. At the end, this systematic review culminated in the sample of 11 documents all in English. The flow chart (adapted from PRISMA diagram) is illustrated down below.

### 3.2 Studies characteristics

In our findings, four articles reported an association of high WP consumption with liver injury as toxicity (Whitt et al. 2008; Gurgen et al. 2014; Nunes et al. 2013; Deminice et al. 2015) and increase their markers of oxidative stress (Deminice et al. 2015), besides that two studies also related to possible kidney problems (Aparicio et al. 2011; Hattori et al. 2017). The

rest of the findings dealt with the negative effects on the microbiota (Moreno-Pérez et al. 2018), acne (Pontes et al. 2013) and expression of anger (Santos et al. 2011).

The age of the studies participants varied from 20 to 45 years old. Four studies included physical activity practitioners, only one article included menopausal women, one included healthy individuals, the rest of the articles selected were with animals, all rats. Regarding the studies design, of the 11 papers that made this systematic review, the most prevalent among humans' studies was randomized trials (4), then, one descriptive observational, and we decided to use one case-control study because of its relevance to the theme of this paper, the remaining ones were experimental, as already mentioned. Table 2 summarizes the articles that made up this review.

## 4. Discussion

### 4.1 Safety use of protein supplementation

Proteins are biologically active substances which have several functions in the body, making up hormones, enzymes and cellular structures (Lobley et al. 2000; Hulmi et al. 2009). The use of a protein supplementation can be beneficial during the moment of the physical exercise to maintain the preservation of muscle mass and avoid proteolysis. Daily protein requirements are determined by the ability to maintain a positive balance between degradation and synthesis (Pitkanen et al. 2003; Tang et al. 2007).

According to an international consensus the daily reference intake of protein for the healthy adult population is 0.8 g/kg body weight (Campbell et al. 2007; Phillips and van Loon 2011), people who practice physical activity may have their needs increased, ranging from 1.2 to 2 g/kg of body weight (Thomas et al. 2016). It is known that WP is the most commonly used protein supplement as a strategy to increase protein intake, mainly by athletes and physical activity practitioners (Cribb 2005; Knoesen et al. 2009; Hida et al. 2012).

The ideal dose of WP that an individual should consume varies according to their goals, level of physical activity and current body composition (Areta et al. 2013; Breen and Phillips 2013). However, some studies indicate that a dose between 20 and 25 g/day of WP is sufficient to achieve the proposed benefits whereas doses above 40 g/day related to possible adverse effects on the organism (Witard et al. 2014).

**4.2 Effects on kidney function**

The studies of Aparicio et al. (2011) and Hattori, Tiselius, and Heilberg (2017) found in this review associated the consumption of a hyperproteic diet with the appearance of kidney problems, using, respectively, animal and human models. Aparicio et al. (2011) showed that consumption of a diet supplemented with a short-term WP intake increased plasma urea, urinary volume and urinary calcium excretion while decreasing pH and urinary citrate. Decreased urinary pH concomitantly with hypocitraturia and hypercalcemia are recognized as a risk for nephrolithiasis (Amanzadeh et al. 2003; Pak 2008). Though, this result was not found in the Aparicio's study.

Regarding renal function, the main concern is a possible overload of the kidneys due to an increase in the pressure and rate of glomerular filtration from a hyperproteic diet, since urea, the main product of protein metabolism, is excreted by this organ (Bankir et al. 1996; Heilberg and Goldfarb 2013). However, a systematic review by Martin et al. (2005) has shown that this renal hyperfiltration can be an adaptive mechanism and, therefore, would not cause damage to the organism of healthy individuals.

Another systematic review, in this case of experimental studies, points out that the use and supplementation of WP does not alter the kidney biomarkers, such as creatinine and urea, and does not change histological tissue on renal glomeruli and tubules as well (Carlos et al. 2019).

**4.3 Effects on liver function**

In the works using animal models of Deminice et al. (2015) and Gurgen et al. (2014), excessive use of WP without exercise was associated with liver problems, presenting elevated alanine aminotransferase (ALA) and aspartate aminotransferase (AST), enzymes associated with liver injury (Kaneko et al. 2008). Also, in Nunes et al. (2013) study, the sedentary group that consumed WP had those same results, but, in contrast, the group that exercised and used WP, the expression of liver injury markers decreased. It appears that a high protein intake in the lack of necessity for the body or sedentary lifestyle can cause detrimental effects on liver function (Blomstrand et al. 2006).

The relationship of hyperproteic diets with the onset of liver problems is interrelated with increased stress promoted by excess liver proteins (Rodriguez et al. 2009). The amino acids that make up the proteins have in their structure a carbon skeleton and a nitrogenous part, at the moment of their metabolization, these structures must be separated, a process that can happen in the liver called deamination (Lobley et al. 2000). The nitrogenous part results in ammonia which is converted into urea through the urea cycle which also occurs in the liver, eventually going to the kidneys where it will be excreted (Welle et al. 1995; Lobley et al. 2000).

In the presence of physical activity, the amino acids (AA) circulating in the bloodstream after the consumption of WP are used by the muscle to increase the protein synthesis and phosphorylation of the mammalian target of rapamycin (mTOR) (Paim et al. 2014). A high rate use of amino acids by the muscle determines less metabolization of them by the liver, as well as lower urea formation (Kanda et al. 2013). Therefore, a protein supplementation associated with exercise more substrate will be provided to the muscle and less to the liver (Bankir et al. 1996; Frank et al. 2009).

We have to mention that some studies found that AA can stimulate mTORC1 in skeletal muscle without exercise (Cao et al. 2019, Norton et al., 2012), and exercise itself also stimulates

muscle protein synthesis through mTORC1-independent mechanisms such as eIF2B dephosphorylation (Gordon et al. 2013).

A very interesting case study found in this review correlate the use of protein supplementation with hepatic injuries. A healthy young male patient developed a deep hepatic cholestasis associated with the presence of jaundice without any type of biliary obstruction or hemolysis due to prolonged use of protein supplements, being WP and creatine (Whitt et al. 2008). It is important to emphasize that some studies regarding individuals with liver damages, such as chronic hepatitis and non-alcoholic hepatic steatosis (NASH), WP intake has shown to be beneficial in reducing inflammation (Chitapanarux et al. 2009; Elattar et al. 2010).

**4.4 Effects on the intestinal microbiota**

It is known that the diet has a direct influence on the intestinal microbiota modification, both positively and negatively (Lopez-Legarrea et al. 2014). Physical activity can also modify the microbiota by changing intestinal transit as well as promoting the growth of some beneficial bacteria (Bressa et al. 2017). In the study of Moreno-Perez et al. (2018), which evaluated the microbiota of athletes supplemented with WP, there was a decrease in some beneficial bacteria, as well as an increase in the growth of malefic bacteria, the *Bacteroides phylum*. It is important to report that other works found in the literature positively associated the consumption of WP with benefits in the microbiota with increase of intestinal *Bifidobacterium* and *Lactobacillus* (Meddah et al. 2001; Swiatecka et al. 2011). The difference between the studies may be due to several factors, such as time and amount of WP used.

**4.5 Other relevant consequences on health**

The works of Santos et al. (2011) and Pontes et al. (2014) related, respectively, chronic protein supplementation to increased expression of anger and acne in physical activity practitioners. The two authors associated the nutritional composition of WP as a justification for their findings.

It is important to highlight that both studies had some limitation regarding the methodological aspects. In Santos' work, anger was measured by a survey of bodybuilders regarding frequency and volume of supplemental protein intake, including non-WP supplements. He didn't account for other correlated factors that could be important, such as preparing for competition (Andersen et al., 1995). Furthermore, Pontes' study has no control group for the protein supplement treatment. It is reported that there was a progression of acne lesions after 2 months of supplementation. We cannot know if a control group, without supplementation, would have experienced different results.

Serotonin is the main neurotransmitter linked to impulsivity and depression, with tryptophan (TRP) being an essential amino acid in its synthesis (Rossi and Tirapegui 2005). It is known that branched-chain amino acids (BCAAs) present in the WP can compete with the TRP in the passage of the blood-brain barrier, thus reducing the synthesis of serotonin and may cause the symptoms related to anger (Kapczinski et al. 2003; Rossi et al. 2003). On the other hand, in the study of Thomson et al. (2011), anger was reduced by 25% in physically active humans after supplementation of leu-rich proteins (including free Leu milk protein powder and WP isolate). Therefore, the papers are still unclear about what mechanisms are behind those results.

In milk and its derivatives, there is the presence of insulin-like growth factor 1 (IGF-1), a compound associated with the promotion of the growth and division of cutaneous cells, production of sebum, and increase of estrogen factors linked to the appearance of acne (Adebamowo et al. 2005; Melnik 2011). Although WP does not have IGF-1, some products containing WP may have it. Thus, this may be one of the explanations why high consumption of WP products are related to the appearance of acne lesions.

**4.6 Primary outcomes**

The use of WP in satisfactory doses seems to be beneficial as the problem being its high consumption, especially in the long term. Moreover, in the studies found, the dose, type of WP

used (isolated, hydrolyzed or concentrated) as time of use varied widely, it is not possible to say in fact if these factors influenced to the results of the works somehow. Despite of the limited number of articles found, we managed to conduct a systematic review that dealt with the association of WP consumption with the presence of possible deleterious effects on the body. As far as we know, no other review was made regarding this subject.

## 5. Conclusion

This review of the literature has found some studies that related the chronic use in high doses of WP with different negative effects to the organism, mainly in sedentary individuals. It was observed that the dose and time of consumption are closely related to the adverse effects of the use of WP. Therefore, consumption of WP above requirement has led to adverse outcomes in some cases and beneficial outcomes in others. However, some studies have not been able to differentiate whether the negative effect was exclusively due to the use of WP or excess of proteins in general, and the literature is scarce on papers in this perspective. Therefore, it would be interesting to propose future research to evaluate the difference and effects on the body comparing a hyperproteic diet, without supplementation, with one supplemented with WP. In addition, research that also deals with the use in different doses and distinct populations and its relation with the organism, both in short and long term.

## Conflicts of interest

The authors have no conflicts of interest to report.

## Authors contributions

The study was designed and written by VASCONCELOS; data were analysed by BACHUR and ARAGÃO; data interpretation and manuscript preparation were undertaken by

VASCONCELOS, BACHUR, and ARAGÃO. All authors approved the final version of the paper.

**Acknowledgements**

We thank the Group of Studies in Neuroinflamation and Neurotoxicology (GENIT), of the Ceará State University for the support in this work.

**References**

Adebamowo CA, Spiegelman D, Danby FW, Frazier AL, Willett WC, Holmes MD. 2005. High school dietary dairy intake and teenage acne. J Am Acad Dermatol. **52**:207–14. doi: 10.1016/j.jaad.2004.08.007.

Aimutis WL. 2004. Bioactive properties of milk proteins with particular focus on anticariogenesis. J Nutr. **134**:989–95. doi: 10.1093/jn/134.4.989S

Allen LH, Haskell M. 2002. Estimating the potential for vitamin a toxicity in women and young children. J Nutr. **132**:907–19. doi: 10.1093/jn/132.9.2907S.

Amanzadeh J, Gitomer WL, Zerwekh JE, Preisig PA, Moe OW, Pak CY, Levi M. 2003. Effect of high protein diet on stone-forming propensity and bone loss in rats. Kidney Int. **64**:2142–149. doi: 10.1046/j.1523-1755.2003.00309.x.

Andersen AE, Woodward PJ, Lafrance N. 1995. Bone mineral density of eating disorder subgroups. Int J Eating Disord. **18**:49-50. doi: 10.1002/1098-108x(199512)18:4<335::aid-eat2260180406>3.0.co;2-t.

Aparicio VA, Nebot E, Porres JM., Ortega FB, Heredia JM, Lopez-Jurado M, Ramirez PA. 2011. Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats. Br J Nutr. **105**:836–45. doi: 10.1017/S0007114510004393.

Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, Jeacocke NA, Moore DR, Stellingwerff T, Phillips SM, et al. 2013. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. J Physiol. **591**:2319–31. doi: 10.1113/jphysiol.2012.244897.

Bankir L, Bouby N, Trinh-Trang-Tan MM, Ahloulay M, Promeneur D. 1996. Direct and indirect cost of urea excretion. Kidney Int. **49**:1598–1607. doi: 10.1038/ki.1996.232.

Blomstrand E, Eliasson J, Karlsson HKR, Kohnke R. 2006. Branched-chain amino acids activate key enzymes in protein synthesis after physical exercise. ASN. **1**:269–73. doi: 10.1093/jn/136.1.269S.

Borsheim E, Tipton KD, Wolf SE, Wolf RR. 2002. Essential amino acids and muscle protein recovery from resistance exercise. Am J Physiol. **283**:648–57. doi: 10.1152/ajpendo.00466.2001.

Breen L, Phillips SM. 2013. Interactions between exercise and nutrition to prevent muscle waste during ageing. Br J Clin Pharmacol. **75**:708–15. doi: 10.1111/j.1365-2125.2012.04456.x.

Bressa C, Bailen-Andrino M, Perez-Santiago J, Gonzalez-Soltero R, Perez M, Montalvo-Lominchar MG, Mate-Munoz JL, Dominguez R, Moreno D, Larrosa M. 2017. Differences in gut microbiota profile between women with active lifestyle and sedentary women. PLoS ONE. **12**:171–352. doi: 10.1371/journal.pone.0171352.

Campbell B, Kreider RB, Ziegenfuss T, La Bounty P, Roberts M, Burke D, Landis J, Lopez H, Antonio J. 2007. International Society of Sports Nutrition position stand: protein and exercise. J Int Soc Sports Nutr. **4**:8–10. doi: 10.1186/s12970-017-0177-8.

Cao Y, Liu S, Abassi IHR, Cai C, Yao J. 2019. Molecular mechanisms relating to amino acid regulation of protein synthesis. Nutr Res Rev. **32**:183–191. doi: 10.1017/S0954422419000052.

Chitapanarux T, Tienboon P, Pojchamarnwiputh S, Leelarungrayub D. 2009. Open-labelled pilot study of cysteine-rich whey protein isolate supplementation for non-alcoholic steatohepatitis patients. J Gastroenterol Hepatol. **24**:1045–50. doi: 10.1111/j.1440-1746.2009.05865.x.

Cowley SA, Whittaker K, Grigulis A, Malone M, Donetto S, Wood H, Morrow EM. 2013. Why Health Visiting? A review of the literature about key health visitor interventions, processes and outcomes for children and families. National Nursing Research Unit (NNRU). King's College. London, 1–47. Available from: http://www.kcl.ac.uk/nursing/research/nnru/publications/index.aspx.

Cribb PP. 2005. Whey Proteins in Sports Nutrition. Arlington (TX): US Dairy Export Council, I.

Deminice R, Comparotto H, Jordao AA. 2015. Whey protein supplementation increases methionine intake but not homocysteine plasma concentration in rats. Appl Physiol.Nutr Metab. **40**:46-50. doi: 10.1139/apnm-2014-0175.

Denham BE. 2011. Dietary supplements–regulatory issues and implications for public health. JAMA. **306**:428–9. doi: 10.1001/jama.2011.982.

Elattar G, Saleh Z, El-Shebini S, Farrag A, Zoheiry M, Hassanein A, El-Ghannam M, Shendy S, El-Dabaa E, Zahran N. 2010. The use of whey protein concentrate in management of chronic hepatitis C virus – a pilot study. Arch Med Sci. **6**:748–55. doi: 10.5114/aoms.2010.17091.

Frank H, Graf J, Amann-Gassner U, Bratke R, Daniel H, Heemann U, Hauner H. 2009. Effect of short-term high-protein compared with normal-protein diets on renal hemodynamics and associated variables in healthy young men. Am J Clin Nutr. **90**:1509–16. doi: 10.3945/ajcn.2009.27601.

Giannopoulou I, Noutsos K, Apostolidis N, Bayios I, Nassis GP. 2013. Performance level affects the dietary supplement intake of both individual and team sports athletes. J Sports Sci Med. **12**:190–6. doi: 10.3390/nu11092120.

Gordon BS, Kelleher AR, Kimball SR. 2013. Regulation of Muscle Protein Synthesis and the Effects of Catabolic States. Int J Biochem Cell Biol. **45**:2147–2157. doi: 10.1016/j.biocel.2013.05.039.

Gurgen SG, Yucel AT, Karakus AC, Cecen D, Ozen G, Kocturk S. 2014. Usage of whey protein may cause liver damage via inflammatory and apoptotic responses. Hum Exp Toxicol. **28**:1–11. doi: 10.1177/0960327114556787.

Haraguchi FK, Pedrosa ML, Paula H, Santos RC, Silva ME. 2009. Influence of whey protein on liver enzymes, lipid profile and bone formation of hypercholesterolemic rats. Rev Nutr. **22**:517—25. doi: 10.1590/S1415-52732009000400007.

Hattori CM, Tiselius HG, Heilberg IP. 2017. Whey protein and albumin effects upon urinary risk factors for stone formation. Urolithiasis. **45**:421–8. doi: 10.1007/s00240-017-0975-0.

Heilberg IP, Goldfarb DS. 2013. Optimum nutrition for kidney stone disease. Adv Chronic Kidney Dis. **20**:165–174. doi: 10.1053/j.ackd.2012.12.001.

Hida A, Hasegawa Y, Mekata Y, Usuda M, Masuda Y, Kawano H, Kawano Y. 2012. Effects of egg white protein supplementation on muscle strength and serum free amino acid concentrations. Nutrients. **4**:1504–17. doi: 10.3390/nu4101504.

Huang S, Johnson K, Pipe A. 2006. The use of dietary supplements and medication by Canadian athletes in the Atlanta and Sydney Olympic Games. Clin J Sport Med. **16**:27–33. doi: 10.1097/01.jsm.0000194766.35443.9c.

Hulmi JJ, Kovanen V, Selanne H, Kraemer WJ, Hakkinen K, Mero AA. 2009. Acute and long-term effects of resistance exercise with or without protein ingestion on muscle

hypertrophy and gene expression. Amino Acids. **37**:297–308. doi: 10.1007/s00726-008-0150-6.

Kanda A, Nakayama K, Fukasawa T, Koga J, Kanegae M, Kawanaka K, Higuchi M. 2013. Post-exercise whey protein hydrolysate supplementation induces a greater increase in muscle protein synthesis than its constituent amino acid content. Br J Nurt. **7**:1–7. doi: 10.1017/S0007114512006174.

Kaneko JJ, Harvey JW, Michael LB. 2008. Clinical Biochemistry of Domestic Animals: San Diego (CA): Elsevier Inc. p. 10, VI.

Kapczinski F, Quevedo J, Izquierdo I. 2003. Bases biológicas dos transtornos psiquiátricos: Porto Alegre (RS): Artmed. p. 10, I.

Knoesen N, Thai Vo S, Castle D. 2009. To be superman – the male looks obsession. Aust Fam Physician. **38**:131–33. PMID: 19283253.

Laure P, Binsinger C. 2005. Adolescent athletes and the demand and supply of drugs to improve their performance. J Sport Sci Med. **4**:272–77. PMID: 24453531.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C., Ioannidis, J.P.A., et al. 2009. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. PLoS Med. **6**:1-10. doi: 10.1371/journal.pmed.1000100.

Lobley GE, Milano GD, Walt JG. 2000. The liver: integrator of nitrogen metabolism: Wallingford. p. 149–168, I.

Lopez-Legarrea P, Fuller NR, Zulet MA, Martinez JA, Caterson ID. 2014. The influence of Mediterranean, carbohydrate and high protein diets on gut microbiota composition in the treatment of obesity and associated inflammatory state. Asia Pac J Clin Nutr. **23**:360–68. doi: 10.6133/apjcn.2014.23.3.16.

Machado J.CC, Navarro F, Navarro AC. 2019. Systematic review about the effect of whey proteins on the renal function of rats and mice in relation to creatinin, ureia, proteinuria, and renal glomerules and tubules. RBNE. **13**:749-807. ISSN 1981-9927. Available in: http://www.rbne.com.br/index.php/rbne/article/view/1438.

Martin WF, Armstrong LE, Rodriguez NR. 2005. Dietary protein intake and renal function. Nutr Metab. **2**:25. doi: 10.1186/1743-7075-2-25.

Meddah AT, Yazourh A, Desmet I, Risbourg B, Verstraete W, Romond MB. 2001. The regulatory effects of whey retentive from bifidobacteria fermented milk on the microbiota of the simulator of the human intestinal microbial ecosystem (SHIME). J Appl Microbiol. **91**:1110–17. doi: 10.1046/j.1365-2672.2001.01482.x.

Melnik BC. 2011. Evidence for acne-promoting effects of milk and other insulinotropic dairy products. Nestle Nutr Workshop Ser Pediatr Program. **67**:131–45. doi: 10.1159/000325580.

Moreno-Perez D, Bressa C, Bailen M., Hamed-Bousdar S, Naclerio F, Carmona M, Perez M, Gonzalez-Soltero R, Montalvo-Lominchar MG, Carabana C, et al. 2018. Effect of a protein supplement on the gut microbiota of endurance athletes: A randomized, controlled, double-blind pilot study. Nutrient. **10**:337–53. doi: 10.3390/nu10030337.

Norton LE, Wilson GJ, Layman DK, Moulton CJ, Garlick PJ. 2012. Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. Nutr Metab. **9**:67–75. doi: 10.1186/1743-7075-9-67.

Nunes R, Silva P, Alves J, Stefani G, Petry M, Rhoden C, Dal Lago P, Schneider CD. 2013. Effects of resistance training associated with whey protein supplementation on liver and kidney biomarkers in rats. Appl Physiol Nutr Metab. **38**:1166–69. doi: 10.1139/apnm-2013-0004.

Paim JA, Lopes AL, Ferreira TR, Conceicao MCA. 2014. Síntese proteica muscular e influência da suplementação de ômega 3: aspectos atuais. Revista Destaques Acadêmicos. **6**:80–8. Available in: http://www.univates.br/revistas/index.php/destaques/article/view/418.

Pak CY. 2008. Pharmacotherapy of kidney stones. Expert Opin Pharmacother. **9**:1509–18. doi: 10.1517/14656566.9.9.1509.

Pasiakos SM, McLellan TM, Lieberman HR. 2015. The effects of protein supplements on muscle mass, strength, and aerobic and anaerobic power in healthy adults: A systematic review. Sports Med. **45**:111–31. doi: 10.1007/s40279-014-0242-2.

Phillips SM, Van Loon LJ. 2011. Dietary protein for athletes: from requirements to optimum adaptation. J Sports Sci. **29**:29–38. doi: 10.1080/02640414.2011.619204.

Pitkanen HT, Nykanen T, Knuutinen J, Lahti K, Keinanen O, Alen M, Komi PV, Mero AA. 2003. Free amino acid pool and muscle protein balance after resistance exercise. Med Sci Sports Exerc. **35**:784–92. doi: 10.1249/01.MSS.0000064934.51751.F9.

Pontes TC, Fernandes FGM, Trindade AS, Sobral Filho JF. 2013. Incidence of acne vulgaris in young adult users of protein-calorie supplements in the city of João Pessoa – PB. An Bras Dermatol. **88**:907–12. doi: 10.1590/abd1806-4841.20132024.

Rodriguez NR, DiMarco NM, Langley S. 2009. Nutrition and athletic performance. Position stand of the American College of Sports Medicine: American Dietetic Association and Dieticians of Canada. Med Sci Sports Exerc. **41**:709–31. doi: 10.1249/MSS.0b013e31890eb86.

Rossi L, Castro IA, Tirapegui J. 2003. BCAA supplementation and changes in cerebral serotonin concentration. Nutrire Rev Soc Bras Aliment Nutr. **26**:1–10. Available in: https://pesquisa.bvsalud.org/controlecancer/resource/pt/lil-365194.

Rossi L, Tirapegui J. 2005. Serotonina e a Neuromodulação Alimentar. Nutrição em Pauta. **13**:36–40. Available in: https://www.nutricaoempauta.com.br/lista_artigo.php?cod=37.

Santos JFS, Maciel FHS, Menegetti D. 2011. Consumption of protein supplements and anger expression in bodybuilders. J Phys Educ. **22**:623–35. doi: 10.4025/reveducfis.v22i4.13166

Swiatecka D, Dominika S, Narbad A, Ridgway KP, Kostyra H. 2011. The study on the impact of glycated pea proteins on human intestinal bacteria. Int J Food Microbiol. **145**:267–72. doi: 10.1016/j.ijfoodmicro.2011.01.002.

Tang JE, Manolakos JJ, Kujbida GW, Lysecki PJ, Moore DR, Phillips SM. 2007. Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men. Appl Physiol Nutr Metab. **32**:1132–138. doi: 10.1139/H07-076.

Thomas DT, Erdman KA, Burke LM. 2016. American college of sports medicine joint position statement. Nutrition and Athletic Performance. Med Sci Sports Exerc. **48**:543–68. doi: 10.1249/MSS.0000000000000852.

Thomson JS, Ajmol A, Rowlands DS. 2011. Leucine-protein supplemented recovery feeding enhances subsequent cycling performance in well-trained men. Appl Physiol Nutr Me. **36**: 242-253. doi: 10.1139/h10-104.

Vasconcelos QDJS, Bachur TPR, Aragao GF. 2018. Whey protein: Composition, use and benefits – A narrative review. EJPESS. **4**:173-183. doi: http://dx.doi.org/10.46827/ejpe.v0i0.1405.

Wakefield AP, House JD, Ogborn MR, Weiler HA, Aukema HM. 2011. A diet with 35% of energy from protein leads to kidney damage in female Sprague-Dawley rats. Br J Nutr. **106**:656–63. doi: 10.1017/S0007114511000730.

Walter P. 2001. Towards ensuring the safety of vitamins and minerals. Toxicol Lett. **120**:83–7. doi: 10.1016/s0378-4274(01)00286-7.

Walzem RL, Dillard CJ, German JB. 2002. Whey components: millennia of evolution create functionalites for mammalian nutrition: what we know and what we may be overlooking. Crit Rev Food Sci Nutr. **131**:1956–72. doi: 10.1080/10408690290825574.

Welle S, Thornton C, Statt M. 1995. Myofibrillar protein synthesis in young and old human subjects after three months of resistance training. Am J Physiol Renal Physiol. **268**:422–27. doi: 10.1152/ajpendo.1995.268.3.E422.

Whitt KN, Ward SC, Deniz K, Liu L, Odin JA, Qin L. 2008. Cholestatic liver injury associated with whey protein and creatine supplements. Semin Liver Dis. **28**:226–31. doi: 10.1055/s-2008-1073122.

Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. 2014. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. Am J Clin Nutr. **99**:86–95. doi: 10.3945/ajcn.112.055517.

Wong AW, Magnuson BA, Nakagawa K, Bursey RG. 2011. Oral sub chronic and genotoxicity studies conducted with the amino acid, L-glutamine. Food Chem Toxicol. **49**:2096–102. doi: 10.1016/j.fct.2011.05.023.

Yoshizawa F. 2004. Regulation of protein synthesis by branched-chain amino acids in vivo. Biochem Biophys Res Commun. **313**:417–22. doi: 10.1016/j.bbrc.2003.07.013.

## Appendices

### 1. Table 1

**Table 1.** Description of the PICO criteria used in human studies (Population, Intervention, Comparison, Outcome, and Studies) for systematic review.

| PICO Criteria | Description |
|---|---|
| Population | Adults (20–45 years of age), Physical activity practitioners (17–45 years of age), both sexes, and Women after menopause (50–65 years of age) |
| Intervention | Whey Protein (varied doses) |
| Comparison | Control Groups |
| Outcome | Chronic and abusive use of whey protein may cause adverse effects on health, especially on kidneys and liver |
| Studies | Randomized crossover, Prospective observational, and only one Case-Control |

## 2. Table 2

**Table 2.** Summary of data extracted from articles found.

| Author(s) / Year | Dose | Timing of Treatment | Species / Sex | Key findings |
|---|---|---|---|---|
| MORENO-PEREZ et al., 2018. | It ranged from 1.8-3 g/kg WP alone. | Ten weeks. | Cross-Country Male Athletes. | - Increased protein intake.<br>- Presence of relevant negative modification in the microbiota.<br>- There was no change in the concentrations of SCFAs, ammonia, pH and water content in fecal samples. |
| PONTES et al., 2013. | It varied according to participants. Not specified. | Sixty days. | Practitioners of Physical Activity of Both Sexes. | - Increased appearance and/or exacerbation of acne lesions.<br>- The result was more relevant in females and in those who had no history of acne. |
| SMITH et al., 2018. | Group with normal protein content: 0.8 g/kg body weight.<br><br>Group with high protein content: 1.2 g/kg body weight. | Six Months | Women after Menopause. | - After losing 5 to 10% of body weight there were no adverse effects on maximum muscle strength, with only a small decrease in muscle mass.<br><br>- Increased protein intake by more than 50% had no significant clinical therapeutic effect on muscle gain or strength. |
| WHITT et al., 2008. | Not specified. | Four weeks. | Male | - Case study of a patient who had chronic use of creatine and WP for four weeks prior to the appearance of acute renal failure and jaundice<br>- Viral hepatitis, genetic and autoimmune diseases have been ruled out.<br>- The pathology was induced by protein supplements, WP being most evident due to the recent association of its use with the appearance of symptoms. |
| HATTORI; TISELIUS; HEILBERG, 2017. | 27 g/day (independent of weight) of WP alone. | Three days. | Men and Women. | - Elevation of protein nitrogen content.<br>- Individual 50% increase in urinary calcium was observed in 39% of supplemented individuals.<br>- Decreased urinary pH by 44%.<br>- There was no change in the values of calcium, phosphorus, sodium, potassium magnesium, uric acid, citrate, oxalate, creatinine, pH and urinary saturation indices between groups. |
| SANTOS; MACIEL; MENEGETTI, 2011. | 3.33 g/kg body weight (mean). | Minimum of six months (varied according to | Men Practicing Physical Activity. | - Elevation of anger scores considered higher than usual in the group that used protein supplementation. |

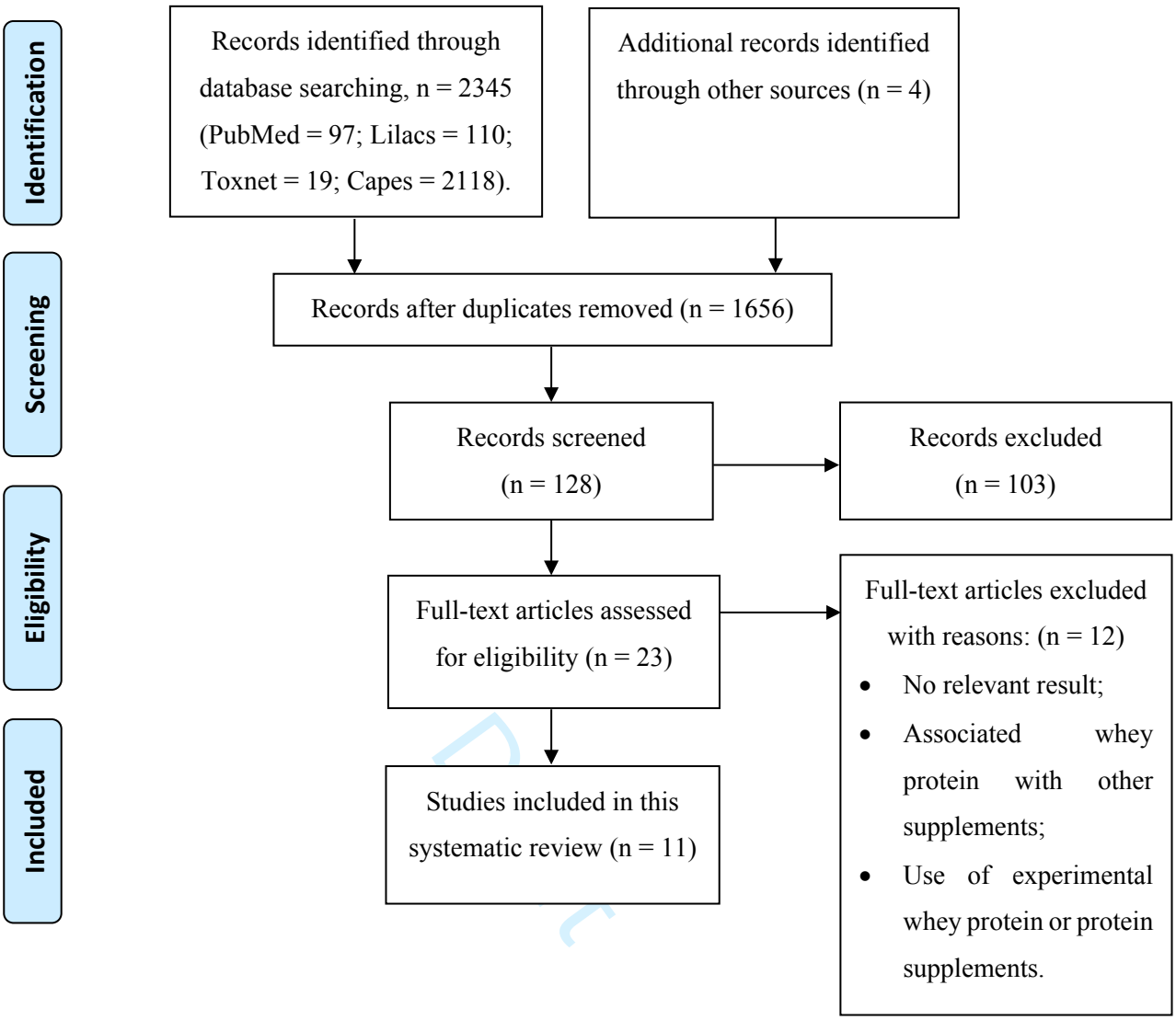
| | | | | |
|---|---|---|---|---|
| | | the participant). | | |

**Table 2.** (Contd.)

| | | | | |
|---|---|---|---|---|
| DELAMAIRE et al., 2012. | Normal protein content: 8.7 g/dL.<br><br>High protein content: 13 g/dL. | Fifteen Days. | Male Sprague-Dawley Rats. | - Low weight in the neonatal period.<br>- Weight gain in puberty and adulthood.<br>- Increase in food intake.<br>- Increase serum insulin, leptin and triglycerides.<br>- Increased number of pancreatic β-cell and the size of adipocytes.<br>- Presence of mesenteric fat mass. |
| DEMINICE, COMPAROTTO, JORDAO, 2015. | Dose free of a diet with 20% of the caloric value coming from proteins. | Four weeks. | Male Wistar Rats. | - Increased intake levels of methionine and protein.<br>- Increased SAM and SAH levels.<br>- No change in Hcy values.<br>- Increased biomarkers of oxidative stress in the liver.<br>- Elevated levels of IL-1β in the WL group. |
| GURGEN et al., 2014. | 252 g/kg body weight. | WS: Five days.<br><br>WL: Four weeks. | Male Wistar Rats. | - IL-6 and TNF-α were decreased in the WS group and elevated in the WL group.<br>- Significant change in ALT in the WL group.<br>- High presence of inflammatory infiltration around the portal area in the liver in the WL group. |
| NUNES et al., 2013. | 1.8 g/kg body weight. | Eight weeks. | Male Wistar Rats. | - Elevation in plasma of creatinine, ALP, AST and ALT in the group supplemented with WP without physical activity. |
| APARICIO et al., 2011. | Group with normal protein content: 13.8 g/100g diet.<br><br>Group with high protein content: 63.6 g/100g diet. | Three Months | Male Wistar Rats. | - Increased kidney weight, urinary volume and acidification.<br>- Increased calcium excretion in parallel with reduced excretion of citrate.<br>- No deleterious effects were found on bone tissue.<br>- Physical exercise had protective effect against renal changes and some metabolic parameters. |

**Note:** WP: whey protein; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine; Hcy: Homocysteine; WS: Group supplemented briefly; WL: Group supplemented for a long time; IL: Interleukin; TNF: Tumor necrosis factor; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; SCFAs: Short-chain fatty acids.

**Figure 1.** Study identification flow chart.


Identification
Screening
Eligibility
Included
Records identified through database searching, n = 2345 (PubMed = 97; Lilacs = 110; Toxnet = 19; Capes = 2118).
Additional records identified through other sources (n = 4)
Records after duplicates removed (n = 1656)
Records screened (n = 128)
Records excluded (n = 103)
Full-text articles assessed for eligibility (n = 23)
Full-text articles excluded with reasons: (n = 12)
No relevant result;
Associated whey protein with other supplements;
Use of experimental whey protein or protein supplements.
Studies included in this systematic review (n = 11)