International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

Role of hyper whey and super amino 2500 body building protein in impairment of thyroid structure and function

Doaa A. Ali[1], Osama A Moftah[2], Hassan IH El-Sayyad[1]*

[1]Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt,
[2] Omar Al Mukhtar University, Derna, Libya
*Corresponding author: *elsayyad@mans.edu.eg*

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript. The author(s) declare that they have no competing interests.

Abstract

Athletes and bodybuilder supplemented high protein supplements to enhance the development and strength of their muscles and obtaining a lot of energy. However, there is no available of work concerned thyroid dysfunction in men intake high protein supplements In the present work, we selected three animal groups of male Wistar albino rats (n=5), control, hyper whey (2.5g/kg body weight) and super amino 2500 (2.5g/kg body weight). Daily oral intake was carried out for 14 weeks. At the end of experiment, animals were sacrificed, dissected and blood collected from puncture of heart and thyroid glands were removed. Plasma triiodothyronine (T3) and thyroxine (T4) were determined. Histopathological and histochemical investigation of glycogen and proteins were assayed. Hyper whey and super amino supplementations were found to show apparent depletion of T3 and non-significant decrease of T4 coincides with considerable atrophy and degeneration of thyroid follicles, missing of lumina colloid and marked loss of follicular epithelium. Finally the author concluded that intake of the high protein diet led to disruption of thyroid hormone parallel with histopathological abnormalities of thyroid follicles which may interfere with the reproductive function. Finally the authors concluded that high whey and super amino protein intake led to damage of thyroid follicles confirmed by depletion of T3 & T4.

Keywords: Anabolic body building protein, Thyroditis, T3&T4, Histopathology, Histochemistry of glycogen and protein.

Background

Most athletes and bodybuilders use high protein diet supplements to enhance the development and strength of their muscles (Lemon, 1991).Casein, whey, and soy proteins are the most popular protein supplements, having a high caloric value and increases muscle mass. Whey protein contains total cow's milk protein (approximately 20%), lactoglobulin (50%), B_lactalbumin (25%), serum albumin (7%), and immunoglobulins (5%). Due to the high concentrations of amino acid , it plays a significant role in the protein synthesis and carbohydrate metabolism giving energy requirements during exercise (Yoshizawa, 2004). Alarming widespread use of anabolic and amino acid supplements has been associated with pathological abnormalities such as abdominal pain, nausea , vomiting, hypercalcemia, elevated liver enzymes and high levels of amylase, lipase and creatine protein kinase (Samaha et al., 2008). Excessive protein intake may lead to dehydration, gout, liver and kidney damage, calcium loss, and gastrointestinal disturbances (Beltz and Doering , 1993). Intake of high protein diets was found to cause a considerable increase in kidney weight, urinary volume and acidity, as well as in the urinary excretion of Ca, with a parallel reduction in the urinary excretion of citrate leading to alterations in

renal health status in pig (Jia et al.,2010) rats (Aparicio et al., 2011). Feeding mice with a 50% casein diet led to a marked increase in serum ALT) and AST activities , liver Finkel-Biskis-Jinkins (FBJ) osteosarcoma oncogene and nuclear receptor subfamily 4, group A, member 1 mRNA levels (Oarada et al., 2012).High protein/high meat intake in humans were associated with the development of disorders such as bone and calcium homeostasis, renal dysfunction, increased cancer risk, liver dysfunction, and progression of coronary artery disease (Delimaris et al., 2013). There is no available work on thyroid glands. The present study aimed to illustrate the role of consuming of two anabolic protein supplements on structural and function of thyroid gland of male albino Wistar rats.

Methods:

1. Hyper whey-treatment:

Hyper whey protein matrix ultra-filtered high quality produced by Nutrabolics, Richmond (B.C. Canada) was used. It is composed of maltodextrin, glycine, cocoa, natural and artificial flavours, cellulose gum, colour, sucralose as well as optimal balance of amino acids especially glutamine or glutamic acid and taurine. Each rat received daily oral doses of 2.5g/kg body weight in drinking water for 14 weeks.

2. Super amino2500-treatment

Super amino 2500 is a highly efficient protein source produced by APN, Launderale (FL 33301, U.S.A). It is composed of a mixture of amino acids Each rat received daily oral doses of 2.5g/kg body weight in drinking water for 14 weeks.

3. Experimental Work:

Thirty fertile male Wistar albino rats weighing approximately 60g body weight, obtained from Hellwan Breeding Farm, Ministry of Health, Egypt were used for experimentation. Free access of standard diet composed of Protein 21.27%, Fat 2.83% and Fiber 2.46% was supplied. Water was allowed *ad-libitum.* They were kept in good ventilation with 12 hour light and dark cycle. The rats were arranged into three groups (n=10) ; Control , fed on standard diet, Hyper whey-treated group (HW) and Super amino 2500-treated group (SA) fed on standard diet plus supplemented the anabolic protein. During intervals of treatment, body weights were recorded every 2 weeks interval till 14 weeks. At the end of treatments, the rats of both control and experimental groups were anesthetized by diethyl ether and sacrificed. Blood was collected by puncture from heart in clean tubes containing EDTA anticoagulants, centrifuged at 3000 rpm and plasma collected. Liver was separated and processed for the following investigations:

1.Histological investigations & morphpmetric assessments:

Thyroid glands were incised immediately, fixed in 10% phosphate-buffered formalin (pH 7.4) and processed for histological investigations. Serial 5 µm thick sections were cut and stained with hematoxylin and eosin, Periodic acid schiff staining (PAS) for glycogen and bromophenol blue for protein [13 [(Drury and wallington,1967) and examined under bright field microscope.

2.Determination of Triiodothyronine (T3) and thyroxine (T4):

Plasma Triiodothyronine (T3) and thyroxine (T4) were determined according the instruction of measured by BioVision'Triiodothyronine (T3) (Mouse/Rat) ELISA Kit(Cat #. K7422-100). The level of T3 is decreased in hypothyroid patients and is increased in hyperthyroid patients. BioVision's mouse/rat Triiodothyronine (T3) kit is a solid phase competitive ELISA Kit. The samples, and T3 enzyme conjugate are added to the wells coated with anti-T3 and anti-4 polyclonal antibody. Unbound T3 and T3 enzyme conjugate are removed. The colour develops after addition of the substrate and its intensity is measured spectrophometrically at wave length 450 nm. .A standard curve is prepared and the concentration of the T3 is determined.

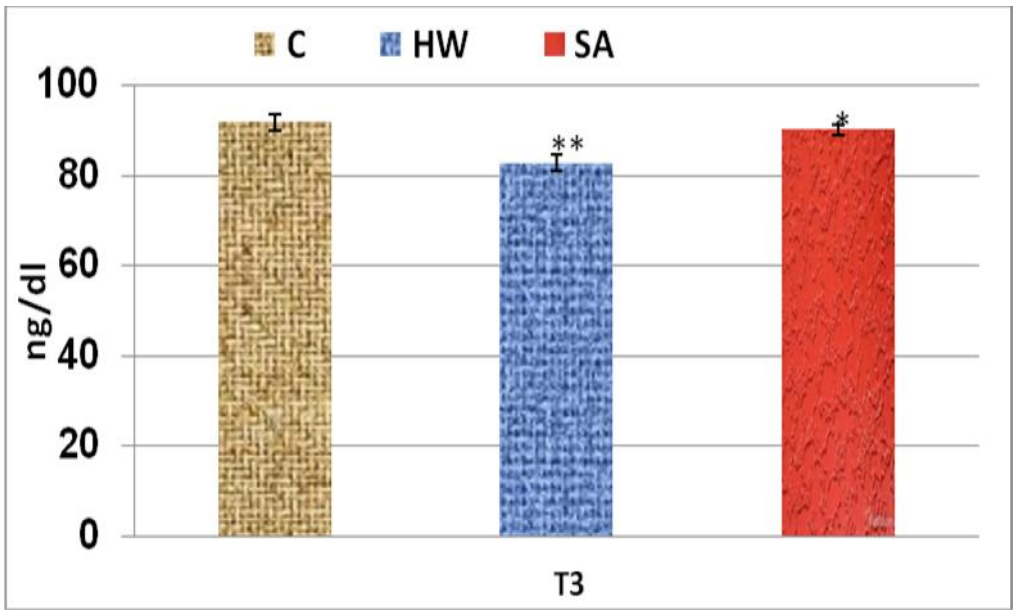
Results

Plasma levels of T3 &T4:

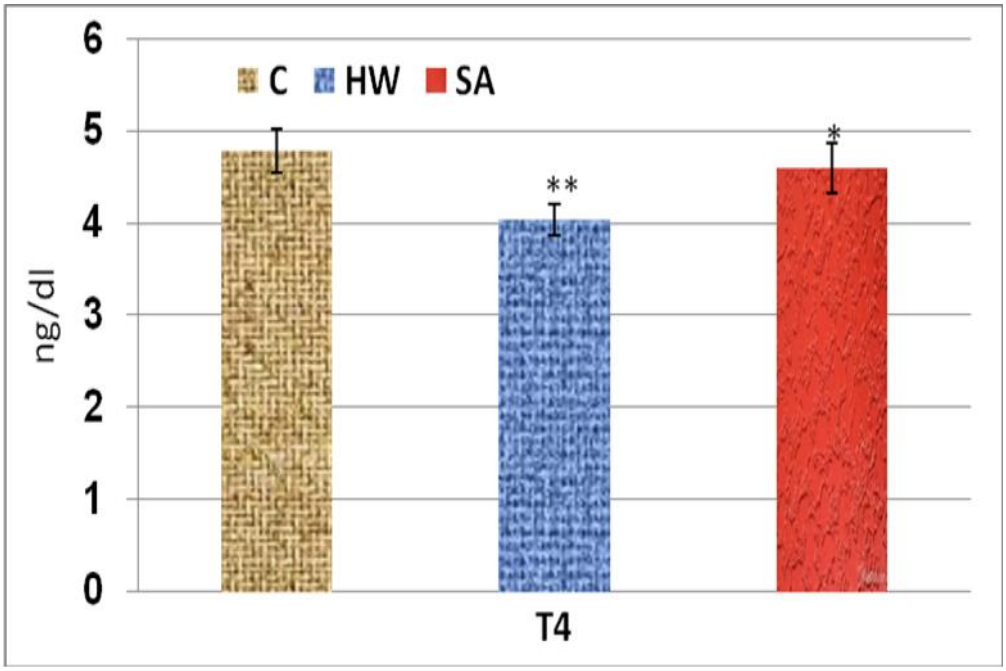
Hyper whey and SA supplementation caused a significant decrease of T3 and non-significant decrease of T4 in comparison with the control (Fig.1 A& B)

Histopathology & Histochemistry:

Control rats exhibited the presence of follicles of varying sizes outlined by cubical follicular cells with rounded vesicular nuclei. Their lumina were enclosed by homogenous eosinophilic colloid material. The interfollicular cells and blood capillaries occupied the space between thyroid follicles. The follicles were separated by thin collagen fibers (Fig. 1A).



A. T3 (ng/dl)



B. T4(ng/dl)

Fig. 1 A&B. T3(A) and T4(B) level of male rats supplemented hyper whey and super amino 2500 for 14 weeks. Each result represent the mean±SE (n=5). * means significant at P<0.05; ** means non-significant at P<0.05. Abbreviation; C ,Control; HW ,Hyperwhey; SA, Super amino 2500.

In experimentally group supplemented either hyper whey of super amino 2500, there was a detected apparent congestion of the follicles with marked reduction of their luminal size in many of the follicles. Meanwhile other showed either vacuolated colloid missing of the colloid material. Few numbers of the thyroid follicles exhibited exfoliation of their lining cells within their follicular lumina. Interfollicular haemorrhage and congested blood vessels were markedly remarked. The follicular epithelium appeared either vacuolated or entirely degenerated. Super amino supplementation revealed massive degenerative phases (Fig. 2 A-C3).

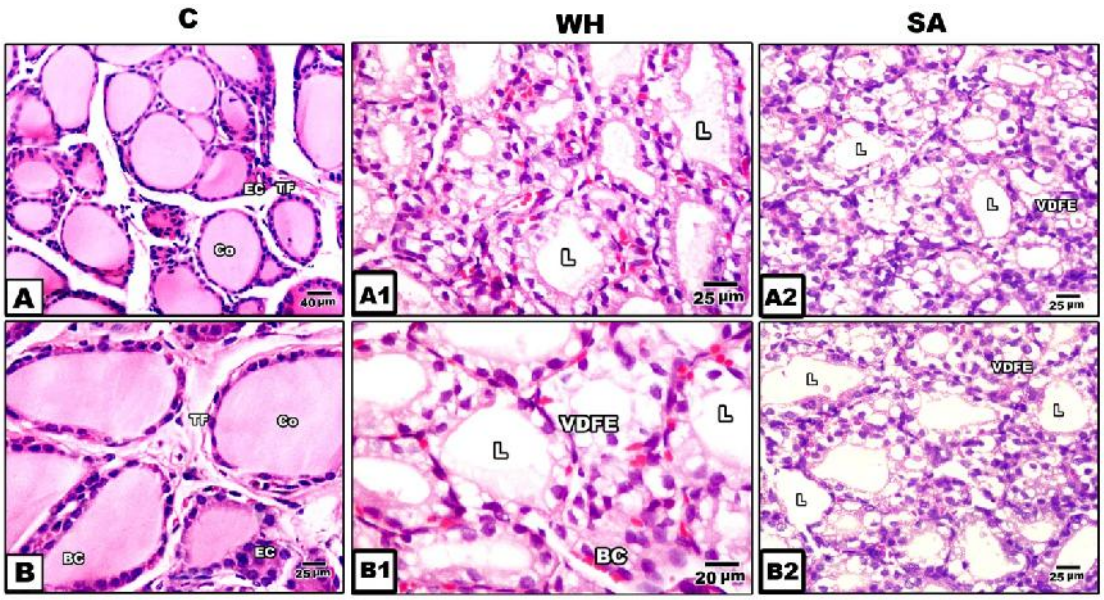


Fig. 2. Photomicrographs of histological section of thyroid gland of male rats. A-B. Control showing normal follicles outlined with follicle epithelium which enclose internal eosinophilic lumen containing colloid. A1-B1. Hyper whey-supplemented group showing vacuolar degenerated follicle epithelium and missing colloid, B1-B2. Super amino 2500 supplemented group massive degeneration of thyroid follicles. Abbreviation; BC, blood capillary; Co, colloid; EC, epithelium cells; L, lumen; TF, thyroid follicle ;VDFE, vacuolar degenerated follicles epithelium. H&E.

Concerning PAS staining affinity, control showed intense PAS staining in both the follicular lumen and their epithelial lining cells (Fig.2 A&B). However, HW &SA supplemented groups revealed faint PAS staining affinity in the damaged follicle, meanwhile other ones are comparatively reduced (Fig.3 A1-B1 & A2-B2).

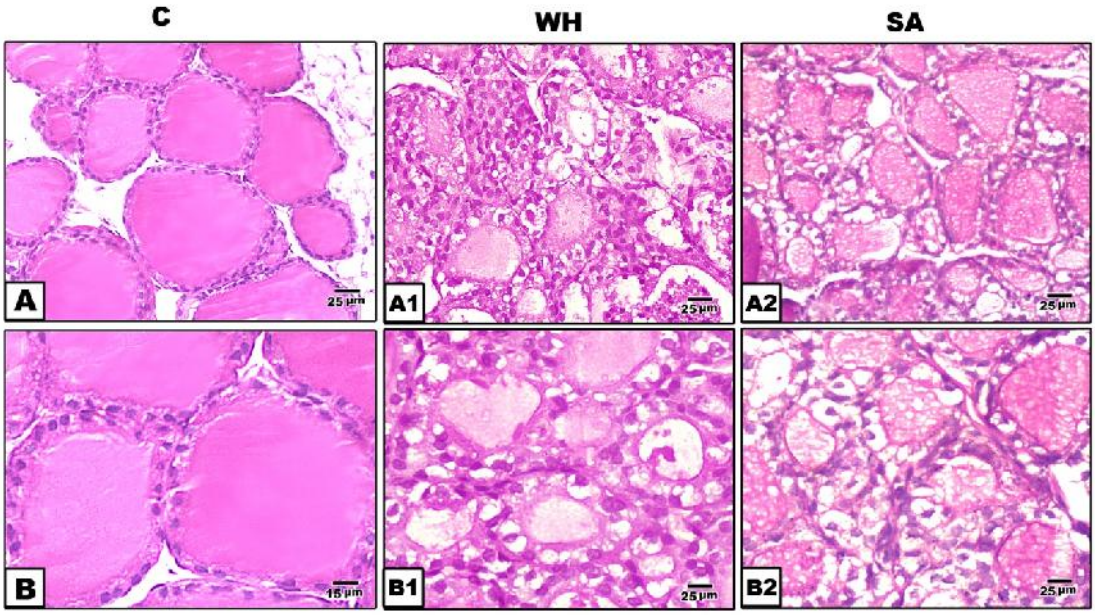


Fig. 3. Photomicrographs of histological section of thyroid gland of male rats. A&B. Control showing dense PAS staining in follicle epithelium and thyroglobulin. A1&A2. Hyper whey-supplemented group showing glycogen depletion. B1 & B2. Super amino 2500-supplemented group showing apparent depletion of glycogen. PAS staining

Bromophenol-blue staining thyroid follicles showed increased staining affinity on both thyroid follicle epithelium and their lumina of the control group (Fig. 4A). On the other hand, HW & SA supplemented group exhibited apparent depletion of the protein staining affinity in the thyroid follicular epithelium and lumina of the follicles (Fig. 4 B1-C!).

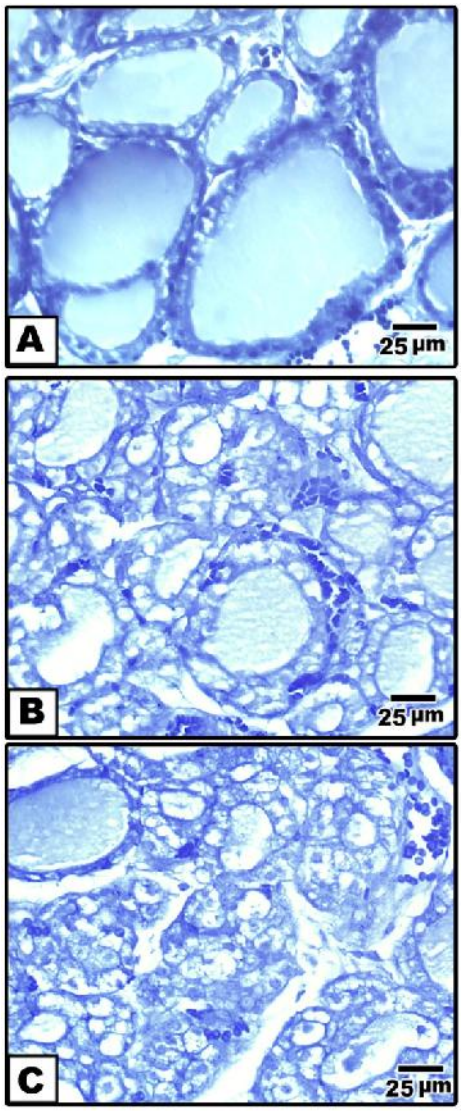


Fig.4 photomicrographs of histological section of thyroid gland of male rats stained by bromophenol blue showing comparatively decreased protein content in thyroid epithelium and follicular lumen of hyper whey (B) and super amino 2500(C) in comparison with the control (A). Mercury-bromophenol blue.

Discussion

Most athletes and bodybuilders use protein supplements to enhance the development and strength of their muscles as well as giving the energy requirements during exercise (Yoshizawa, 2004). However, there is no available work concerning thyroid dysfunction.

The present findings revealed that rat supplemented daily 2.5g/kg body weight of hyper whey or super amino 2500 for 14 lead to marked reduction of the size of thyroid follicle and vanishment of their colloid material coincides with vacuolar degeneration of thyroid follicle epithelial cells. Interfollicular haemorrhage and congested blood vessels were markedly detected. Thyroid gland of the treated rats showed marked depletion of glycogen and protein contents representing mainly in follicle epithelium and colloid material.

Many authors such as Huang and Liaw (1995), Gardner et al. (2011), Penteado et al. (2015) reported that there is a correlation between liver disease and thyroid disorder.

Anabolic steroid hormones were found to reduce thyroxine binding globulin (TBG), and total serum T3 and T4 (Alèn et al.,1987; Fortunato et al.,(2007;).

Malik and Hodgson (2002) mentioned that T3 & T4 play a great role in the metabolism of hepatocytes and consequently promote hepatic function. Disruption of thyroid function may lead to liver disease and vice versa.

Alteration of liver transaminases was reported in men (Pettersson et al., 2008) as well as in experimental rat (Jean et al., 2001; Morfuji et al., 2005) and mice (Delimaris, 2013) fed on high protein diet.

Degeneration of thyroid follicle cells and deformed lumina of the follicles were confirmed by depletion of plasma T3 level in both hyper whey and super amino 2500 ,however T4 non-significantly decreased in both treatment.

Similar findings were recorded by Deyssig and Weissel (1993) in young (20-29 yr old) male body builders recieved androgen-anabolic steroids exhibited apparent reduction of T3 & T4.

Rats received Soya protein supplementation exhibited significant depletion of T3 & T4. These was associated with a depletion of hepatic mitochondrial respiration with succinate substrate (Klein et al., 2000). The thyroid function (T3 & T4) was markedly reduced by the administration of anabolic-androgenic steroids (AAS) decadurubolin (DECA) (Fortunato et al.,2010).

It is well known that T3 and T 4 are synthesized and secreted by thyroid follicle cells. T3 is the most biologically active hormone and most of them formed from T4 after deiodination in liver (Gupta et al.,1997). Serum levels of thyroid hormones, including T3 and T4 are markers of the thyroid function in humans and experimental animals. Changes in their concentration may reflect dysfunction in their glandular synthesis and secretion (Kelly, 2000).

Finally the authors concluded that high whey and super amino protein intake led to damage of thyroid follicles confirmed by depletion of T3 & T4.

References

Alèn M, Rahkila P, Reinilä M, Vihko R (1987) Androgenic-anabolic steroid effects on serum thyroid, pituitary and steroid hormones in athletes. Am J Sports Med 15(4):357-361.

Amanzadeh J, Gitomer WL, Zerwekh JE, Preisig PA, Ow M, Pak CYC, Levi M (2003) Effect of high protein diet on stone-forming propensity and bone loss in rats. Kidney Int 64(6):2142-9.

Aparicio VA, Nebot E, Porres JM, Ortega FB, Heredia JM, Lopez-Jurado M, Ramırez PA(2011) Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats. Br J Nutr 105(6):836-45.

Beltz SD, Doering PL (1993) Efficacy of nutritional supplements used by athletes. Clin Pharm 12(12): 900-908.

Delimaris I (2013) Adverse Effects Associated with Protein Intake above the Recommended Dietary Allowance for Adults. ISRN Nutr2013:126929.

Delimaris I(2013)Adverse Effects Associated with Protein Intake above the Recommended Dietary Allowance for Adults. ISRN Nutr. 2013:126929.

Deyssig R and Weissel M (1993) Ingestion of androgenic-anabolic steroids induces mild thyroidal impairment in male body builders. J Clin Endocrinol Metab 76(4):1069-1071.

Drury R A, Wallington E A.*Carleton's histological technique.*, Ed. 5.,Oxford University Press, London, England, 1967.

Fortunato RS, Rosenthal D, Carvalho DP (2007) Abuse of anabolic steroids and its impact on thyroid function. Arq Bras Endocrinol Metabol. 51(9):1417-1424.

Fortunato RS, Marassi MP, Chaves EA, Nascimento JH, Rosenthal D, Carvalho DP (2010) Chronic administration of anabolic androgenic steroid alters murine thyroid function. Med Sci Sports Exerc 38(2):256-261.

Gardner CJ, Richardson P, Wong C, Polavarapu N, Kemp GJ, Cuthbertson DJ (2011) Hypothyroidism in a patient with non-alcoholic fatty liver disease. BMJ 342:c7199.

Gupta P, Chaurasia SS, Maiti PK, Kar A (1997) Cadmium induced alterations in extrathyroidal conversion of thyroxine to triiodothyronine by type-I iodothyronine 5'-monodeiodinase in male mouse. Horm Metab Res. 29: 151-152.

Huang MJ, Liaw YF. Clinical associations between thyroid and liver diseases. J Gastroenterol Hepatol. 1995;10(3):344-50.

Jean C, Rome S, Mathé V, Huneau J, Aattouri N, Fromentin G, Achagiotis CL, Daniel Tomé D

(2001) Metabolic evidence for adaptation to a high protein diet in rats. J Nutr 131(1):91-8.

Jia Y, Hwang SY, House JD, Ogborn MR, Weiler HA, Karmin O, Aukema HM (2010) Long-term high intake of whole proteins results in renal damage in pigs. J Nutr. 40(9):1646-1652.

Kelly GS (2000) Peripheral metabolism of thyroid hormones: a review. Altern Med Rev 2000, **5**: 306-333.

Klein M, Schadereit R, Küchenmeister U (2000) Energy metabolism and thyroid hormone levels of growing rats in response to different dietary proteins--soyprotein or casein. Arch Tierernahr 53(2):99-125.

Lemon PW (1991) Protein and amino acid needs of the strength athlete. .Int J Sport Nutr. 1(2): 127-45.

Malik R, Hodgson H (2002) The relationship between the thyroid gland and the liver. Q J Med 95:559–569.

Morifuji M, Sakai K, Sugiura K (2005) Dietary whey protein modulates liver glycogen level and glycoregulatory enzyme activities in exercise-trained rats. Exp Biol Med 230(1): 23–30.

Oarada M, Tsuzuki T, Nikawa T, Kohno S, Hirasaka K, Gonoi T (2012) Refeeding with a high-protein diet after a 48 h fast causes acute hepatocellular injury in mice. Br J Nutr 107(10): 1435–44.

Penteado KR, Coelho JC, Parolin MB, Matias JE, Freitas AC(2015) The influence of end-stage liver disease and liver transplantation on thyroid hormones. Arq Gastroenterol.52(2):124-8.

Pettersson J, Hindorf U, Persson P, Bengtsson T, Malmqvist U, Werkström V, Ekelund M (2008) Muscular exercise can cause highly pathological liver function tests in healthy men Br J Clin Pharmacol 65(2): 253–259.

Samaha AA, Nasser-Eddine W, Shatila E, Haddad JJ, Wazne J, Eid AH (2008) Multi-organ damage induced by anabolic steroid supplements: a case report and literature review. J Med Case Rep 2: 340.

Souza RA, Miranda H, Xavier M, Lazo-Osorio RA, Gouvea HA, Cogo JC, Vieira RP(2009) Ribeiro W. Effects of high-dose creatine supplementation on kidney and liver responses in sedentary and exercised rats. J Sports Sci Med 8(4):672-81

Yoshizawa F (2004) Regulation of protein synthesis by branched-chain amino acids in vivo. Biochem Biophys Res Commun 313(2): 417-22.

Yoshizawa F (2004) Regulation of protein synthesis by branched-chain amino acids in vivo. Biochem Biophys Res Commun. 313(2): 417-22.