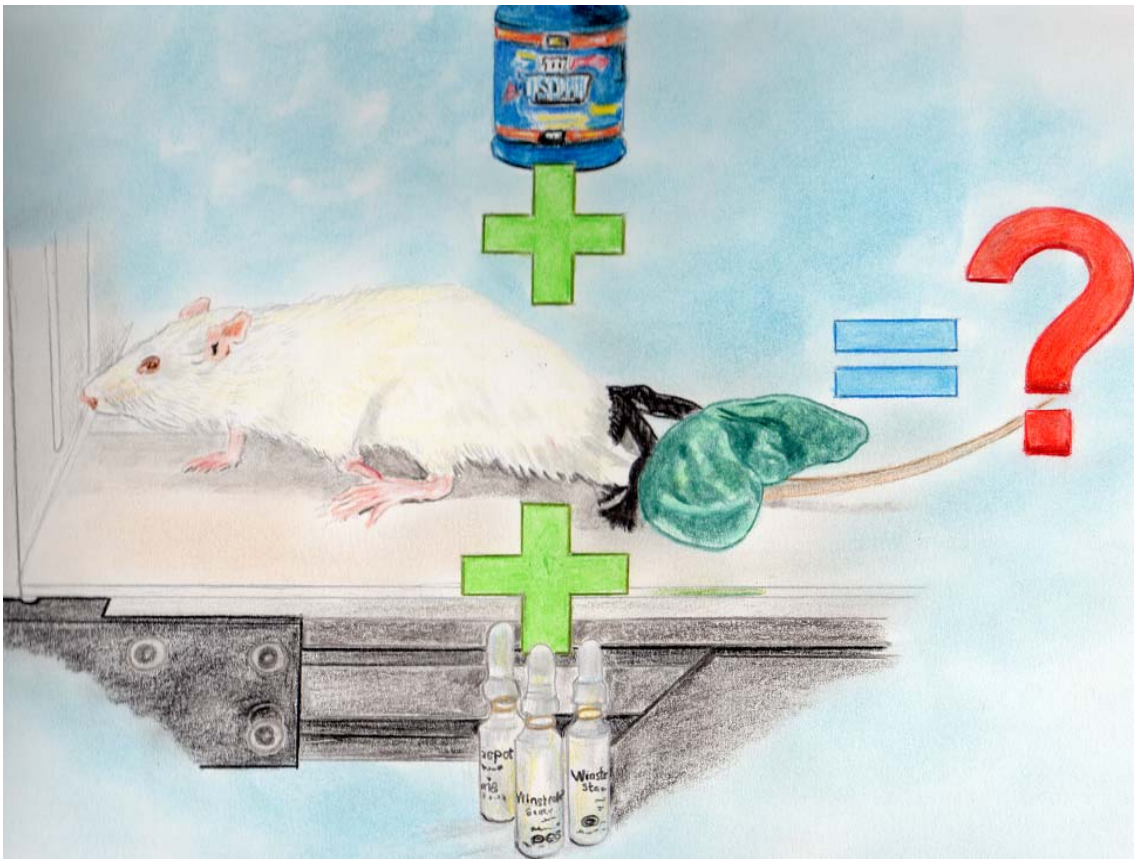
Tesis Doctoral Internacional / International Doctoral Thesis

Efectos del porcentaje y fuente de proteína, del entrenamiento de fuerza y de la administración de esteroides anabolizantes sobre marcadores metabólicos, hepáticos, renales y óseos en ratas

Effects of the amount and source of protein, resistance training and steroids on metabolic, hepatic, renal and bone markers in rats



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VIRGINIA A. APARICIO GARCÍA-MOLINA

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Fdo. Cristina Sánchez González

En Granada, 15 de junio de 2012

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PROYECTOS DE INVESTIGACIÓN [RESEARCH PROJECTS]

El trabajo desarrollado y los artículos que componen la presente memoria de Tesis Doctoral están basados en los siguientes proyectos de investigación:

- **PROYECTO:** Efectos ergogénicos y perjudiciales de los suplementos "whey" valorado sobre un entrenamiento de fuerza en ratas**.** 2007/20SVC.

  ENTIDAD FINANCIADORA: Junta de Andalucía. Consejería de Turismo, Comercio, Ocio y Deporte. Centro Andaluz de Medicina Deportiva.

  FECHA: 31/12/2007 al 31/12/2009

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  INVESTIGADOR PRINCIPAL: Dra. Pilar Aranda Ramírez**.**

- **PROYECTO:** Efectos de esteroides anabolizantes y de una dieta basada en suplementos de lactosuero o proteína vegetal sobre parámetros musculares, hepáticos y renales en ratas sometidas a entrenamiento de fuerza. Acrónimo: "NutriHealth". DEP2008-04376.

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LISTA DE PUBLICACIONES [LIST OF PUBLICATIONS]

La presente memoria de Tesis Doctoral está compuesta por los siguientes artículos científicos:

I. Aparicio VA, Nebot E, Heredia JM, Aranda P. Efectos metabólicos, renales y óseos de las dietas hiperproteicas. Papel regulador del ejercicio. *Revista Andaluza de Medicina del Deporte*. 2010;3(4):153-158.

II. Aparicio VA, Nebot E, Porres JM, Ortega FB, Heredia JM, López-Jurado M, Aranda P. Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats. *British Journal of Nutrition*. 2011;105:836-845.

III. Aparicio VA, Nebot E, Kapravelou G, Porres JM, Sánchez C, López-Jurado M, Aranda P. El entrenamiento de fuerza reduce la acidosis metabólica y la hipertrofia hepática consecuentes del consumo de una dieta hiperproteica en ratas. *Nutrición Hospitalaria*. 2011; 26(6):1500-1508.

IV. Aparicio VA, Sánchez C, Ortega FB, Nebot E, Kapravelou G, Porres JM, Aranda P. Effects of the dietary protein concentration and source, resistance training and anabolic-androgenic steroids on body weight and lipid profile of rats. *Submitted to Nutrition and Metabolism.*

V. Aparicio VA, Nebot E, Tassi M, Camiletti-Moirón D, Porres JM, Sánchez C, Aranda P. Whey vs. soy protein diets and renal status in rats. *Submitted to Nutrition Researh.*

VI. Aparicio VA, Sánchez C, Femia P, Tassi M, Nebot E, Porres JM, Aranda P. Renal effects of high-protein diets, resistance training and anabolic-androgenic steroids in rats. *Submitted to Kidney International.*

RESUMEN

En los últimos años, el consumo de dietas hiperproteicas ha ganado popularidad. Además, en deportistas que llevan a cabo entrenamiento de fuerza hipertrofia, en ocasiones dichas dietas basadas en proteína de lactosuero o soja se combinan con la administración de esteroides anabolizantes.

El objetivo general de esta Tesis Doctoral ha sido analizar los efectos de las dietas hiperproteicas, de la fuente de proteína (lactosuero vs. soja), del entrenamiento de fuerza y de la administración de esteroides anabolizantes sobre marcadores metabólicos, hepáticos, renales y óseos en ratas.

Los principales resultados de la Tesis sugieren que: a) Los marcadores plasmáticos y urinarios estudiados mostraron una mayor tendencia a la acidosis metabólica tras el consumo de la dieta hiperproteica, lo que podría explicar el incremento del peso de hígado y riñón observados y favorecería el riesgo de nefrolitiasis. A pesar de esta mayor acidez, el contenido mineral óseo no se vio afectado. b) El entrenamiento de fuerza tuvo una acción tamponadora sobre el incremento de peso de hígado y riñón ocasionado por la ingesta de dicha dieta hiperproteica. c) El entrenamiento de fuerza incrementó el contenido mineral óseo, estimado mediante el peso en cenizas del fémur. d) De entre todas las intervenciones testadas, la administración de esteroides anabolizantes fue el factor que más negativamente afectó al perfil lipídico plasmático y hepático, mientras que las dietas hiperproteicas y especialmente el entrenamiento de fuerza podrían derivar en un mejor perfil lipídico, especialmente cuando se combinan. No se han observado resultados consistentes que sustenten que el consumo de la proteína de soja sea más favorable sobre la pérdida de peso o el perfil lipídico que la proteína de lactosuero. e) El incremento de la acidez y calciuria urinaria observados en la dieta de lactosuero podría constituir un ambiente más favorable para la formación de cálculos renales que la proteína de soja. Sin embargo, no se han observado diferencias morfológicas en el riñón atendiendo a la fuente de proteína. f) Las dietas hiperproteicas y los esteroides anabolizantes aumentaron el peso del riñón y el área glomerular, especialmente cuando se combinaron. El entrenamiento de fuerza-hipertrofia redujo el peso del riñón y el área glomerular, pero ocasionó un incremento paralelo de la fibrosis renal y tubular.

Los resultados de la presente memoria de Tesis ponen de manifiesto que de entre todas las intervenciones testadas (porcentaje y fuente de proteína, entrenamiento de fuerza y esteroides anabolizantes), el entrenamiento de fuerza se presentó como la mejor terapia promoviendo la pérdida de peso y mejorando, en general, el perfil lipídico. No obstante, bajo nuestra condiciones experimentales, dicho protocolo de fuerza hipertrofia indujo una menos favorable morfología renal.

SUMMARY

In the last years, diets based on high protein intakes have won in popularity. Furthermore, resistance trainers sometimes combine such whey or soy protein based diets with the administration of anabolic androgenic steroids.

The overall objective of this Thesis has been to analyze the metabolic, hepatic, renal and bone effects of high-protein diets, the protein source (whey vs. soy), resistance training and anabolic androgenic steroids in rats.

The main findings from this Thesis suggest that: a) Plasma and urinary markers showed higher metabolic acidity after a high whey or soy protein diet consumption, which could explain the heavier kidney and liver observed in those groups and could increase the risk of kidney stones. Despite of this acidosis, bone mineral content was not affected. b) Resistance training was effective at enhancing bone mineral content, as measured by femur ashes weight. The effects of training were generally observed at the second and third month, suggesting a mid-long term effect. c) Resistance training had a protective action against hepatic and renal inflammation promoted by the high protein diet. d) Among all the interventions tested, anabolic androgenic steroids administration was the factor that most negatively affected plasma and hepatic lipid profile, whereas high-protein diets and hypertrophy resistance training could induce, in general, a better lipid profile, especially when combined. Any consistent benefits on body weight loss, hepatic and plasma lipid profile have been observed derived from soy protein instead to whey-protein consumption. e) The increase of acid and urinary calcium excretion due to the whey-protein diet can constitute a favorable environment for kidney stones and renal diseases. However, any significant renal morphological effects attending to the protein source have been observed. f) High-protein diets and anabolic androgenic steroids increased kidney weight and glomerular area, especially when combined. Hypertrophy resistance training reduced the higher kidney weight observed in those groups and the glomerular area, but with the parallel increase of renal and tubular fibrosis. The high intensity of the training protocol performed under our experimental design might be on the basis of this worse morphological renal status.

The results of the present Thesis highlight the usefulness of resistance training among all the interventions tested (high-protein diets, whey or soy protein source, exercise and anabolic androgenic steroids) on promoting weight loss and improving plasma and hepatic lipid profile, but the present hypertrophy resistance training protocol performed under our experimental conditions induced a less favorable renal morphology.

ABREVIATURAS [ABBREVIATIONS]

AAS	Anabolic androgenic steroids
AIN	American Institute of Nutrition
ANOVA	Analysis of variance
Ca	Calcium
CK	Creatine kinase
CKD	Chronic kidney disease
DM	Dry matter
GFR	Glomerular filtration rate
HDL	High density lipoprotein
HP	High protein
LDL	Light density lipoprotein
MANOVA	Multivariate analysis of variance
N	Nitrogen
NP	Normal protein
RM	Repetition maximum
SD	Standard deviation
SEM	Standard error of the mean
TAG	Triglycerides
TC	Total cholesterol

INTRODUCCIÓN [INTRODUCTION]

1. High protein diets

The use of high protein (HP) diets is a controversial topic. Resistance trainers receive mixed and confounding messages about the safety of purposely seeking ample dietary protein in their quest for enhanced protein synthesis, improving performance, or maintaining health [1]. The current Dietary Reference Intake for general population is 0.8g/kg body weight per day [2]. Individuals engaged in regular exercise training require more dietary protein than sedentary individuals [3]. Protein intake necessary to support nitrogen (N) balance in strength athletes ranges from 1.2 to 1.7 g/kg body weight per day [3-5]. The International Society of Sports Nutrition Position Stand: *Protein and Exercise* [3] concluded that the concerns regarding the potential unhealthiness of protein intake within the range of 1.4–2.0 g/kg body weight per day, are unfounded in healthy, exercising individuals, and were largely based upon data from non-athletes [1]. However, some studies have reiterated the tendency to HP diets consumption by athletes [6] and its potential health damage [7-9].

Under this context, is relevant to better explore and describe the effects of HP diets on health through latest studies on this topic. (PAPER I).

1.1 Effects of high protein diets on body weight and lipid profile

Obesity and abnormal lipid levels contribute significantly to the risk of coronary heart disease, a major cardiovascular disease and a serious health problem [10]. The guidelines by the National Cholesterol Education Program recommend more active control of serum lipids to reduce the incidence of coronary heart disease [11]. Furthermore, hypertriglyceridemia deserves special attention [11]. Nutritional and dietary therapy, weight loss, exercise, and scientifically proven nutritional supplementation should be used to manage dyslipidemia [10].

In the last few years, the use of HP diets (i.e. "The Dr. Dukan diet") is gaining in popularity among the general population. Indeed, HP diets are increasingly being

recommended as one of the management strategies for weight control in overweight and obese individuals [12-13]. HP diets appear to reduce appetite, energy intake, body weight, and fat deposition at the same time that improve plasma lipid profile [14-18]. This body weight reduction may be attributed to a combined effect of the lower caloric intake exhibited by HP experimental diets, and the higher energy expenditure required by digestion and metabolism of protein foodstuffs. Alterations on body weight and food intake caused by HP diet consumption might be on the basis of the lower plasma total- and HDL-cholesterol, and triglycerides exhibited by HP-fed animals. Furthermore, these lower concentrations of total cholesterol and triglycerides could have a protective effect on cardiovascular and kidney disease [10, 19-20].

1.2 High protein diet and renal health

In view of the high prevalence of obesity, type 2 diabetes, and metabolic syndrome [21], it is important to understand the effect of high levels of protein intake on health. This is particularly important for the kidney, because these patients are characterized by renal hyperfiltration and increased risk of kidney disease [22-24].

Despite the evidence about the weight-loss effect of HP diets, the impact of such diets on renal status still unclear [7, 25-28]. Excessive protein consumption might affect renal health [9]. In particular, HP intake may promote renal damage by chronically increasing glomerular pressure and hyperfiltration. Urea, the major end product of protein metabolism in mammals, is the most abundant solute in urine. Plasma urea concentration and/or globular filtration rate increase when normal rats are fed HP diets [29]. More urea needs to be filtered, either because more of it has to be excreted, or because the efficiency of its excretion is reduced. This hyperfiltration might have deleterious consequences in diseased kidneys [29]. However, it is uncertain whether there is significant evidence to support this relationship in healthy individuals. In fact, some studies suggest that hyperfiltration, the purposed mechanism for renal damage, is a normal adaptative mechanism that occurs in response to several physiological conditions [25]. Protein restriction may be appropriate for treatment of existing kidney disease [26], but in healthy persons, significant evidence for a

detrimental effect of HP intakes on kidney function has not been explored enough [25]. (PAPER II).

Until date, two studies have clearly observed renal affectation; Frank *et al.* [30], detected blood urea nitrogen, serum uric acid, glucagon, natriuresis, urinary albumin, and urea excretion increased significantly with a HP diet in healthy young men, and concluded that more attention should be paid to the potential adverse renal effects of HP diets [30]. Similarly, in the recent study performed in pigs (because of the similarity of their organs with the human's kidneys), after 8 months of experimental period, renal and glomerular volume was a 60-70% higher in the HP group. These higher kidneys also displayed higher histological damage, with a 55% more fibrosis and a 30% more glomerulosis. Furthermore, plasma concentrations of homocysteine and MCP-1 (renal monocyte chemoattractant protein-1) were extremely higher.

Moreover, relative excess of animal protein ingestion (acid load from sulphur-containing amino acids) produces intracellular acidosis [28]. Intracellular acidosis stimulates hypocitraturia, that is often accompanied by hypercalciuria [28]. A decrease in urinary pH, hypocitraturia and hypercalciuria, are recognized risk factors for kidney stone formation, principally by increasing urinary saturation of calcium salts [28, 31].

1.3 High protein diet and bone health

Excessive protein consumption might also affect bone health [32-33]. Acting as a buffer system, bone aids in the modulation of acid-base homeostasis [34] through the release of calcium [35]. In theory, elevated animal protein consumption is acidogenic [32], resulting in increased bone resorption [36]. However, despite it has been demonstrated that the excess of dietary protein from either animal or plant proteins may be detrimental to bone health, its effect will be modified by other nutrients [37]. While it is possible that a prolonged HP intake may lead to increased bone resorption and ultimate bone loss, the current evidence on that issue is limited and conflicting. Some studies have reported a negative effect on bone health in both healthy rats [38-39] and humans [40-41]. Conversely, other HP studies in healthy rodents [14, 31, 35] and humans of

different ages [42-43], did not show any relationship. In fact, a negative relationship between low protein diet consumption and bone mineral content has been reported [44-46].

Urinary excretion of Ca is higher in HP-fed experimental groups. Urinary calcium excretion is strongly related to net renal acid excretion [34, 37]. The catabolism of dietary protein generates ammonium ion and sulphates from sulphur-containing amino acids. Bone citrate and carbonate are mobilized to neutralize these acids, so urinary calcium increases when dietary protein increases [34, 37]. Protein is considered to be a net acid-producing substance and thus a net negative risk factor for bone dissolution. However, the acidogenic role of dietary protein remains being a matter of debate, as it is well established that other food components have a clear impact on acid-base balance [47-48]. There is substantial literature supporting the beneficial effects of HP consumption on skeletal metabolism when such protein is consumed together with adequate calcium, potassium, and other minerals, regardless of the protein source [34, 37]. Pye *et al.* [49], found that lower body weight, fat mass and higher lean body mass were associated with a high mixed protein consumption by rats, but did not find any deleterious effect on bone. The authors concluded that a mixed HP diet containing adequate calcium levels and up to 35% of energy can be deemed safe for long-term bone health.

The effects of HP diet consumption on bone health status appear to be a controversial issue that need further study, with authors either reporting a deleterious influence in healthy humans [40-41] and rats [38-39], or describing no adverse or even protective effects of HP supplements in rats [14, 31, 35, 50] and humans [42-43]. (PAPER II).

1.4 High protein diet and hepatic health

Despite numerous studies have analyzed the hepatic effects of high in fat diets, are almost nonexistent those which have analyzed the effects of HP diets on the liver. Studies in rodents have observed a notable increase in liver weight after the consumption of a HP diet [8, 51]. In the study by Hammond and Janes [8], liver weight, as measured in fresh substance, was analyzed after two weeks of HP diet intake. The authors found a 20% substantially increased fresh weight. On the other hand, HP

diets have been shown to improve hepatic steatosis in rodent models and in high-fat fed humans [52-54]. Therefore, more studies analyzing the influence of HP diets on hepatic lipid profile are required. (PAPER III).

Protein supplements, i.e. above 80% protein concentrates or above 90% protein isolates, have become popular among athletes and people interested in gaining muscle mass [55]. It would be of interest to further examine the effects of high percentage of whey protein commercial supplements consumption on body composition, lipid and hepatic lipid profile as well as some renal and hepatic parameters in a well defined animal model. (PAPER II, III and IV).

2. Resistance training

2.1 Effects of resistance training on body weight and lipid profile

Resistance training has become one of the most popular physical activities in the developed countries and increasing number of gyms have been launch in the last decade. Resistance training can enhance absolute muscular strength, hypertrophy and muscular power [56-57] as well as reduce body fat, lipids (triglycerides, total cholesterol, and LDL-cholesterol) and thus, the consequent risk of cardiovascular disease [10, 58-59]. To note is that not all individuals that follow long periods of exercise lose body weight under ad-libitum intake conditions. In fact, some subjects are resistant to weight loss and may even increase it [60]. However, despite these potential gains of body weight, other beneficial changes on body composition are produced, such as increases in lean mass with reductions in fat mass and body circumferences [61-62].

2.2 Resistance training and hepatic health

Results regarding the effects of exercise on hepatic lipid profile still being scarce or inconclusive. Latest researches are starting to clarify this controversy [63-66]. Aerobic

exercise [63, 67] but especially resistance training [65], may reduce the fat concentration in the human liver at the same time that has shown to reduce the insulin resistance in the adipose and hepatic tissue in obese rats [66]. Johnson *et al.* [67] analyzed the effect of aerobic exercise training on hepatic lipids in 19 sedentary obese men and women. After 4 weeks of aerobic cycling exercise, hepatic triglycerides concentrations were reduced a 21%. The authors concluded that regular aerobic exercise reduces hepatic lipids in obesity even in the absence of body weight reduction. On the other hand, Petridou *et al.* [63] examined the effects of 8 weeks of exercise training on the fatty acid composition of phospholipids and triglycerides in rat liver. The fatty acid composition of liver phospholipids changed with training whereas no significant differences in the fatty acid profile of hepatic triglycerides were found.

Training seems to be more potent on reducing intrahepatic fat in subjects with high levels of fat in the liver in its basal state, such as individuals with non-alcoholic steatosis, type-2 diabetes, or those who are in senescence [65].

2.3 Resistance training and renal health

The effects of exercise on renal status need further study. Exercise appears to reduce kidney inflammation, improve glomerular filtration rate (GFR) and plasma albumin concentrations [19, 68-69]. It is well established that inactivity contributes to chronic kidney disease (CKD) [70]. Exercise improves a number of metabolic factors, reduces blood pressure and insulin resistance, which could preserve renal function [68, 70-71]. The evidence suggests that among CKD patients, the risk of remaining inactive is higher and those patients who are weak can benefit from resistance training interventions [72]. Resistance training could increase nitrogen (N) retention and protein synthesis, ameliorates losses of muscle mass and its function, and consequently alleviates proteinuria, and thus, kidney disease in this profile of weaker population (old population, CKD patients, or weightlessness-exposed subjects) [72-75]. On the other hand, strenuous exercise can result in muscle damage evidenced by increased blood levels of muscle proteins such as creatine kinase (CK), lactate dehydrogenase, and myoglobin [76-77]. Renal function could be impaired when myoglobin becomes concentrated in the kidney tubules [78].

Due to the fact that HP diets consumption is an extended practice among sportsmen that may pose some deleterious effects, especially on renal and hepatic health, the buffering action of resistance training on such potential threads still remains to be deeply tested. (PAPER II, III and IV). Moreover, until date, only two studies [71, 75] have analyzed kidneys morphology after an exercise intervention, and were performed in rats in weightlessness condition or with hypertension. Therefore, the morphological effects of resistance training on kidneys of healthy individuals remain unclear. (PAPER VI).

2.4 Resistance training and bone health

Resistance training benefits on bone mineral content have been largely demonstrated [79-84]. Furthermore, exercise appears to be even more important than diet regarding bone strength because it has a direct effect (i.e. via loading) on bone mass and structural properties, whereas nutritional factors have an indirect effect (i.e. via hormonal factors) [79]. On the other hand, in the study by Bennel *et al.* [85] performed in rats, the authors did not appreciate differences in bone of rats developing a resistance training protocol. Burr *et al.* [83] reported that short periods of interrupted resistance training, with rest periods between them, were a more effective osteogenic stimulus than a single sustained session. Activities involving higher loading rates will also be effective for increasing net bone formation, even if the duration of the activity is short [83]. Jumping exercise has shown to be effective at improving mineral bone density in rats [80-82], but in our opinion, is less reproducible to humans than resistance training, especially for old age groups.

Given the complexity of carrying out long-term interventional studies in human subjects and some ethical issues, animal experimental model are often used to study this specific research questions. Bone is a complex tissue that changes slowly. As such, it is difficult to design and conduct well-controlled nutrition studies in humans to quantify the effect of one nutrient or exercise intervention on bone [44]. In our opinion, additional research with rats would be of interest for assessing long-time effect of HP diets and exercise interventions. The extrapolation of rodent studies to humans is especially useful in bone metabolism, mainly because years, not weeks,

are required to assess bone density change in humans [84]. (PAPER II).

3. Protein source (whey vs. soy protein)

Whey is the liquid remaining after milk has been curdled and strained to remove the caseins (curds). It contains proteins, lactose, vitamins, minerals, and traces of fat. Whey protein, represents 20% of the total protein content of milk and has been reported to have utility in many different applications ranging from effects on bone, muscle, blood, brain, pancreas, immune, cancer, infection, metabolism, wound healing, learning, and aging [86]. Soy protein is a vegetable high quality protein, with a protein digestibility corrected amino acid score of 1; it also has a high arginine/lysine ratio, which is associated with lower insulin secretion compared to animal protein. Afterwards, soy protein contains isoflavones, which act as weak estrogens, inhibiting tyrosine kinase-dependent signal transduction processes and functioning as cellular antioxidants [87-88].

3. 1. Protein source (whey vs. soy) influence on body weight and lipid profile

Omnivores have a significantly higher cluster of cardiovascular risk factors compared to vegetarians, including increased plasma total cholesterol, triglycerides, LDL-cholesterol and serum lipoprotein(a) concentrations and worse ratios of total cholesterol/HDL-cholesterol, LDL-cholesterol/HDL-cholesterol and triglycerides/ HDL-cholesterol [89]. Compared with omnivores, vegetarians have lower n-3 polyunsaturated fatty acid (PUFA) levels in the tissue membrane phospholipids and decreased plasma HDL-cholesterol [89].

The effects of soy (vegetal protein) on serum lipoproteins have been of great interest the last decade. From a critical review of the literature, it appeared that recent studies found positive effects on serum LDL-cholesterol concentrations. It is still not clear whether the claimed hypocholesterolemic effects of soy can be attributed solely to the isoflavones [90]. The new soy-based supplements may therefore play a valuable role in

reducing cardiovascular risk [91-92]. Overall, existing data are inconsistent or inadequate in supporting most of the suggested health benefits of consuming soy protein [92]. The Nutrition Committee of the American Heart Association has assessed 22 randomized trials conducted since 1999 and concluded that isolated soy protein with isoflavones slightly decreased LDL-cholesterol but had no effect on HDL-cholesterol, triglycerides and lipoprotein(a) [92]. Although the contributing factors to these discrepancies are not fully understood, the source of soybeans and processing procedures of the protein or ISF are believed to be important because of their effects on the content and intactness of certain bioactive protein subunits. Some studies have documented potential safety concerns on increased consumption of soy products [90, 92]. Therefore, the exact combination of active ingredients in soy products need to be identified [90].

Several human [93-94] and rodent studies [15-16] have demonstrated the ability of whey protein (animal) to improve body composition (increasing muscle mass and/or reducing body weight gain and adiposity index). In fact, whey protein appears to be especially indicated to avoid overweight and increase insulin sensitivity [15-16]. Recently, Bortolotti *et al.* [95] evaluated the effects of a whey protein supplementation for 4 weeks on intrahepatocellular lipids and fasting plasma triglycerides in obese non diabetic women. Whey protein decreased intrahepatocellular lipids by ~21%, fasting total triglycerides by ~15%, and total cholesterol by ~7%. The authors concluded that whey protein reduces hepatic steatosis and improves plasma lipid profile in obese non diabetic patients, without adverse effects on glucose tolerance or creatinine clearance [95]. (PAPER IV).

3. 2. Protein source (whey vs. soy) influence on renal health

It has been well established that the composition of the diet affects acid-base balance in the body. Soybean protein is low in sulphur amino acids [88] and therefore, some nutritional advantages could be obtained by replacing many animal based foods for soy foods [88]. On the other hand, relative excess of animal protein ingestion (acid load from sulphur-containing amino acids) induce intracellular acidosis [28], which could contribute to the formation of calcium-containing kidney stones [28, 31]. Moreover, in a

recent study, urine acidification has been also associated with various metabolic abnormalities in visceral obesity [96].

The renal effects of soy protein have been widely explored, but the results are controversial and inconclusive. In fact, some studies have found a protective role of soy protein on renal health [97-99] whereas other studies failed to find significant improvements [100-101]. To our knowledge, any study has deeply analyzed the effects of whey protein on renal status. (PAPER V).

4. Anabolic androgenic steroids

Anabolic androgenic steroids (AAS) are synthetic derivatives of testosterone [102-103]. The roles of testosterone and its derivatives which are particularly relevant in athletes are anabolic (stimulation of protein synthesis and inhibition of protein degradation in protein metabolism) [104-105]. The AAS were the first substances identified as doping agents, having ergogenic functions and have been widely used to increase muscle mass and strength in adults [104, 106]. In the last few years AAS are an integral part of the nutritional concept of many athletes to improve fitness, muscle gain and exercise performance [106]. AAS abuse is commonly associated with bodybuilders, weightlifters, and other athletes [106]. The misuse of AAS is a practice done not only by higher-level athletes. Increasing evidence indicates that AAS use is associated with non-athletes and is linked to a broader syndrome of problem behaviors rather than efforts to achieve sporting success [107]. There was a significant spread in the general society such as have been showed in the last epidemiological studies [108-110]. These studies have estimated that at least 3% of young men have used AAS at some point in their lives [111]. Currently, AAS tend to become a public health problem [112]. The desired effects of AAS are accompanied by numerous adverse effects both physiological and psychological level [102, 104, 113-116].

4. 1. Anabolic androgenic steroids effects on body weight and lipid profile

The chronic abuse of AAS results in part in extreme alterations in lipoproteins and

apolipoproteins, especially in reducing HDL-cholesterol levels, representing an atherogenic profile, reflecting an elevated risk for cardiovascular disease [117-120].

The effects of AAS-administration on plasma lipid profile were studied in male body builders who received a weekly intramuscular injection of nandrolone-decanoate (100 mg) or placebo for 8 weeks in a double blind way. AAS induced a ~26% decrease in HDL-cholesterol [119]. Frisch and Sumida [120] studied whether compromised serum lipoprotein concentrations would be manifest in rats receiving testosterone injections over the time course of 7 weeks. No significant differences were observed between groups for serum total cholesterol, triglycerides or LDL-cholesterol concentrations. However, at week 7, serum HDL-cholesterol was significantly lower in the testosterone treated rats, compared with control animals. The author concluded that these results suggest that the lipoprotein profile is not altered until week 7 (our study has been performed in 12 weeks). Further, the only compromised parameter under the conditions of the Frisch and Sumida study is the decrease in serum HDL-cholesterol.

4. 1. Anabolic androgenic steroids effects on renal health

Chronic use of AAS has been known to cause serious adverse effects such as liver disorders, neuropsychiatric disorders, adverse blood lipid profiles, cardiovascular disorders, and renal complications [118, 121-122]. Among these disorders, renal diseases have received less attention, probably because are less frequent among AAS users in comparison to other, more prevalent diseases. However, some studies have observed cases of severe renal disorders among AAS users, especially with elevated or prolonged use [122-124].

A limitation of human studies is represented by the fact that information about the intake of AAS is generally self-reported and it is hardly possible to assess the exact dosage. Furthermore, AAS are often used in combination with other drugs or substances, so it is difficult to separate their toxic effects. Hence, experimental studies conducted on animal models are mandatory given the complexity of carrying out long-term and well-controlled interventional studies on this topic in humans, and

some ethical issues. Until date, most of available evidence come from studies that examined the effect of specific interventions, i.e. focus on just exercise or just on the protein amount in the diet. The combined effect and interactions taking place between the dietary amount and protein source, resistance training and AAS-administration on lipid profile and renal health, has never been explored. (PAPER IV and VI, respectively).

The present thesis involved an important number of rats, allocated in different groups so that the main effects of HP diet, resistance training, the protein source (whey or soy) and AAS-administration, and the interactions taking place between them, provided a good opportunity to comprehensively investigate how these lifestyle factors and behaviors can influence renal, bone and hepatic health as well as dyslipidemia, and the consequent risk of coronary heart disease.

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OBJETIVOS

General:

El objetivo general de esta memoria de Tesis Doctoral fue analizar los efectos metabólicos, hepáticos, renales y óseos de las dietas hiperproteicas, de la fuente de proteína (lactosuero vs. soja), del entrenamiento de fuerza hipertrofia y de la administración de esteroides anabolizantes en ratas, así como las interacciones que tienen lugar entre dichas intervenciones.

Específicos:

- Revisar los últimos estudios que analizaron los efectos renales, metabólicos y óseos de las dietas hiperproteicas y el rol que el ejercicio pudiera tener sobre los parámetros sensibles de ser alterados por dichas dietas. (**Artículo I**).

- Valorar los efectos de una dieta hiperproteica de lactosuero y del entrenamiento de fuerza hipertrofia sobre la composición corporal, el perfil lipídico plasmático, hueso y riñón junto con las posibles interacciones que pudieran tener lugar entre ambas intervenciones. **(Artículo II)**.

- Examinar los efectos del consumo de dietas hiperproteicas sobre el perfil lipídico y parámetros de acidosis metabólica, renales y hepáticos en ratas, así como los efectos del entrenamiento de fuerza hipertrofia sobre dichas variables, y su posible interacción con la dieta. (**Artículo III**).

- Estudiar los efectos de las dietas hiperproteicas, de la fuente de proteína (lactosuero vs. soja), del entrenamiento de fuerza y de la administración de esteroides anabolizantes sobre el peso final y el perfil lipídico plasmático y hepático, así como las posibles interacciones producidas entre dichas intervenciones. **(Artículo IV)**.

- Estudiar los efectos de las dietas hiperproteicas, de la fuente de proteína (lactosuero vs. soja), del entrenamiento de fuerza y de la administración de esteroides anabolizantes sobre marcadores urinarios, plasmáticos e histológicos de estado renal, así como las posibles interacciones producidas entre dichas intervenciones. **(Artículos V y VI).**

AIMS

Overall:

The overall objective of this PhD Thesis was to analyze the metabolic, hepatic, renal and bone effects of high-protein diets, the protein source (whey vs. soy), resistance training, and anabolic androgenic steroids administration in rats, as well as the interactions taking place between the different interventions.

Specifics:

- To review current studies analyzing the metabolic, renal and bone effects of high protein diets and the role that exercise could have in such outcomes. (**Paper I**).

- To examine the effect of high whey protein diets and resistance training on body composition, plasma lipid profile, renal and bone parameters, as well as the interactions taking place between both interventions. **(Paper II)**.

- To study the potentially buffering effect of resistance training against the renal and hepatic inflammation and the metabolic acidosis induced by the intake of a high-protein diet. (**Paper III**).

- To examine the effects of high-protein diets, the protein source, resistance training and anabolic androgenic steroids, on final body weight and plasma and hepatic lipid profile, as well as the interactions taking place between such interventions. **(Paper IV)**.

- To examine the effects of high-protein diets, the protein source, resistance training and anabolic-androgenic steroids on plasma, urinary and morphological renal parameters in rats, as well as the interactions taking place between the different interventions. **(Papers V and VI)**.

MÉTODO [METHODS]

Table 1. Summary table of the methodology used in the current Thesis.

Paper	Design	Main variables	Methods
I. *Efectos metabólicos, renales y óseos de las dietas hiperproteicas. Papel regulador del ejercicio.*	Review	Body composition, metabolic acidosis markers, renal and hepatic markers, bone mineral density, plasma lipid profile.	Review of the main articles referred to the topic and extracted from PubMed, Scopus, Cochrane and Sport Discuss databases.
II. *Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats.*	12 experimental groups (n=8) fed normal-protein (10%) or high-protein (45%) diets for 4, 8 or 12 weeks, with or without resistance training.	Body composition, food intake, renal status markers, bone mineral content, urinary acidity markers, plasma lipid profile.	Ashes estimation, atomic absorption spectrophotometry, pHmetry, plasma biochemical autoanalyzer. Resistance training. Diet formulation and preparation.
III. *El entrenamiento de fuerza reduce la acidosis metabólica y la hipertrofia hepática y renal consecuentes del consumo de una dieta hiperproteica en ratas.*	4 experimental groups (n=8): normal-protein (10%) or high-protein (45%) diets for 12 weeks, with or without resistance training.	Body weight, food intake, metabolic acidosis markers, renal weight, liver weight, biochemical renal and hepatic markers, plasma and hepatic lipid profile.	Atomic absorption spectrophotometry, pHmetry. Plasma biochemical autoanalyzer. Resistance training. Diet formulation and preparation. "Folch" method for liver fat extraction
IV. *Effects of dietary protein concentration, protein source, resistance training and anabolic-androgenic steroids on body weight and lipid profile of rats*	16 experimental groups (n=8-10): normal-protein (10%) or high-protein (45%) diets, whey or soy protein source, with or without resistance training and with or without anabolic androgenic steroids for 12 weeks	Body weight, food intake, plasma and hepatic lipid profile.	Resistance training. Diet formulation and preparation. Anabolic androgenic steroids administration. Plasma biochemical autoanalyzer. "Folch" method for liver fat extraction.
V. *Whey vs. soy protein diets and renal status.* ***VI.*** *Renal effects of high-protein diets, resistance training and anabolic-androgenic steroids in rats.*	16 experimental groups (n=8-10): normal-protein (10%) or high-protein (45%) diets, whey or soy protein source, with or without resistance training and with or without anabolic androgenic steroids for 12 weeks,	Urinary and plasma acidity and renal markers. Renal weight and morphology.	Resistance training. Diet formulation and preparation. Anabolic androgenic steroids administration. Plasma biochemical autoanalyzer. Atomic absorption spectrophotometry, pHmetry. Renal histology.

RESULTADOS Y DISCUSIÓN [RESULTS AND DISCUSSION]

Los resultados y discusión se presentan en la forma en que han sido previamente publicados/sometidos en revistas científicas.

1. Efectos metabólicos, hepáticos, renales y óseos de las dietas hiperproteicas y del entrenamiento de fuerza

(Artículos I, II and III)

I

Efectos metabólicos, renales y óseos de las dietas hiperproteicas. Papel regulador del ejercicio

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Revisión

Efectos metabólicos, renales y óseos de las dietas hiperproteicas. Papel regulador del ejercicio

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RESUMEN

El establecimiento de niveles de referencia proteicos seguros, tanto para la población en general como para los deportistas en particular, sigue siendo a día de hoy, fuente de debate. Parece existir un acuerdo científico acerca de los beneficios de las dietas hiperproteicas (HP) sobre el perfil lipídico plasmático, al mejorar los niveles generales de colesterol y triglicéridos y favorecer la pérdida de peso. Sin embargo, los efectos de las dietas HP sobre parámetros renales y óseos aún desencadenan disparidad de resultados. Hay estudios que consideran la hiperfiltración glomerular renal, ocasionada por el consumo de dietas HP, una respuesta fisiológica adaptativa normal, mientras que otros advierten del mayor riesgo de desarrollar una patología renal de mantenerse altas ingestas proteicas de alto valor acidogénico durante años. Es en el metabolismo óseo donde la controversia es mayor. Existen estudios que evidencian una peor densidad mineral ósea, otros que no encuentran diferencias significativas y otros que atribuyen a las dietas HP un efecto protector óseo.
Tanto en el ámbito metabólico, como en el renal y óseo, el ejercicio físico se presenta como una herramienta reguladora excelente ante la mayoría de las alteraciones que dichas dietas pudieran ocasionar, al fomentar un mejor perfil lipídico, reducir la inflamación renal, mejorar la ratio de filtración glomerular y estimular el fortalecimiento óseo.
Tras demostrarse en el estudio de Elango et al (2009) que las ingestas proteicas diarias recomendadas de 0,8 g/kg/día estaban infravaloradas, y establecerse los nuevos niveles para la población sedentaria, deberían formularse nuevos niveles seguros de referencia de proteína de alto valor biológico para atletas de las distintas disciplinas.

Key words:
High-protein diet.
Exercise.
Kidney.
Bone.
Lipid profile

ABSTRACT

Metabolic, renal and bone effects of high-protein diets. The protective role of exercise

The establishment of safe dietary protein intake reference levels for the general population as well as for athletes is under debate. There is evidence indicating a positive benefit of high protein diets (HP) on total cholesterol and triglycerides, and on promoting weight loss.
The findings on the effect of HP diets on renal and bone metabolism are however contradictory. While there are studies that consider the renal glomerular hyperfiltration, caused by the consumption of HP diets, a normal adaptive physiological response, others find an increased risk of renal disease after chronic HP diets. Regarding bone metabolism, there are studies showing a worse bone mineral density after a HP diet, others that did not observe any effect on bone metabolism, or even a bone protective effect.
Exercise is a key player in most of the HP diets-related effect on metabolic acidity, renal and bone health. There is compelling evidence that exercise positively influence blood lipid profile, renal inflammation and glomerular filtration rate, and stimulating bone mineral content and density.
The study of Elango et al (2009) showed that recommended daily protein intake of 0.8 g/kg/day were undervalued, and thus they established new reference levels for the general population. There is still an urgent need to formulate safe protein intake recommendations for athletes of different disciplines.

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Introducción

Las proteínas deberían aportar aproximadamente tan sólo del 8 al 15% de las calorías totales ingeridas por la persona, con modificaciones ligeras en los deportistas atendiendo al periodo de entrenamiento, precompetición o competición[1,2]. A pesar de que a día de hoy sigue siendo común la creencia de que las proteínas se emplean como fuente energética, sólo excepcionalmente, y siempre de forma poco relevante, se metabolizan como medio de obtención de energía (en una competición de *Iron-Man*, de más de 6 horas de duración, o en situaciones extremas de supervivencia, por ejemplo)[2]. Por ello, a la hora de diseñar dietas y establecer los niveles proteicos adecuados para las distintas poblaciones, se debe priorizar su función plástica y estructural con respecto a la energética.

Nuestro organismo puede sintetizar proteínas a partir de aminoácidos, pero sólo es capaz de producir algunos de estos aminoácidos (aminoácidos no esenciales). Aquellos aminoácidos que no podemos sintetizar (esenciales o indispensables), deben ser aportados necesariamente por la dieta. Esto plantea que los requerimientos no sean estrictamente de proteínas, sino de aminoácidos[1]. Por lo tanto, se deben consumir alimentos proteicos que contengan gran variedad de aminoácidos. Este término es el llamado "valor biológico" de la proteína. Así, los alimentos que contienen proteínas completas o de alto valor biológico, son aquellos que presentan en su composición química todos o la mayoría de los aminoácidos esenciales[1].

La estimación y determinación de niveles proteicos de referencia saludables continúa generando controversia. Los deportistas, y más especialmente aquellos que llevan a cabo entrenamiento de fuerza, siguen recibiendo mensajes diversos acerca de la cantidad y fuente de proteína apropiada para mejorar y estimular la síntesis proteica[3]. Las recomendaciones proteicas actuales de ingestas diarias de referencia (RDI) para la población general se sitúan en torno a 0,8 gramos de proteína por kilogramo de peso corporal y día[4], siempre que sean proteínas de alto valor biológico. Sin embargo, los individuos que desarrollan ejercicio de forma regular requieren una mayor ingesta proteica que aquellos que son sedentarios[5]. Actualmente, se estima como ingesta apropiada para un aporte suficiente de nitrógeno para los sujetos que realizan actividad física de forma activa entre 1,0 y 1,2 g/día por kilogramos de peso corporal en mujeres y de 1,2 a 1,4 g/día por kilogramo de peso corporal en hombres[5-7]. En deportistas que llevan a cabo entrenamiento de fuerza, los rangos recomendados oscilan entre 1,2 y 1,7 g/kg de peso corporal y día[5-7]. Estas cifras pueden elevarse hasta 2 g/día por kilogramo de peso corporal en algunos colectivos especiales de deportistas que por su disciplina deportiva necesiten un desarrollo muscular elevado (halterofilia, lucha, culturismo, etc.), así como también en deportistas sometidos a un gran esfuerzo y desgaste muscular durante largos periodos de tiempo, como los ciclistas profesionales[8].

La Sociedad Internacional de Nutrición Deportiva (ISSN), en su documento de consenso sobre proteínas y ejercicio[5], concluyó que la popular afirmación acerca de que niveles proteicos de entre 1,4 y 2,0 g/kg de peso y día no era saludable, no estaba basada en la evidencia científica ni en individuos que llevasen a cabo ejercicio físico regular, sino en años de estudio en personas sedentarias[3] y que, por lo tanto, dichos niveles de proteína no suponían ningún riesgo renal, óseo, hepático o metabólico al individuo. A pesar de esto, cabría destacar que es una práctica habitual entre los deportistas recurrir a ingestas proteicas excesivas, tanto por parte de deportistas de alto nivel, como de aficionados, ya sea en deportes individuales como de equipo[9,10]. El consumo proteico suele estar muy por encima del recomendado, principalmente en deportistas de especialidades anaeróbicas y deportes donde predomina la capacidad de fuerza y desarrollo muscular, como puede ser el culturismo o la lucha[11], en los que se llega a ingerir en algunas ocasiones hasta 5 g/día por kilogramo de peso corporal[8]. Este fenómeno en ocasiones acontece por el desconocimiento nutricional de los deportistas y entrenadores, ya que si aumenta mucho el total de calorías ingeridas (lo cual es normal para personas físicamente activas y más si cabe para deportistas con muchas horas de entrenamiento al día), la proporción de energía en forma de proteínas debe tender a disminuir[11]. En el campo del culturismo se ha extendido la idea de que una elevada ingesta de proteínas, ya sea ingiriendo grandes cantidades de huevos y carnes, ya sea a través de suplementos deportivos proteicos o de complejos de aminoácidos, ocasiona un aumento de la masa muscular.

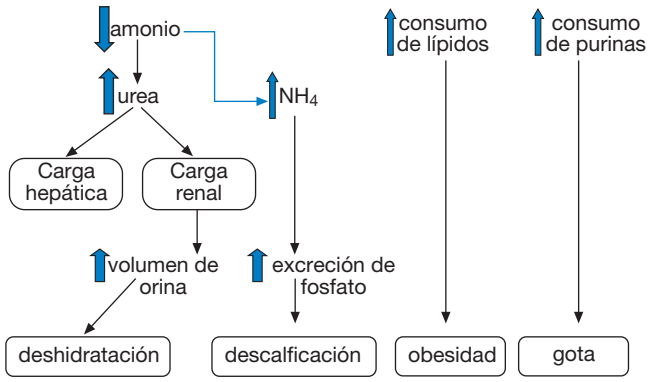

Fig. 1. Posibles efectos adversos de una dieta hiperproteica. Tomado de Barbany[2].

Hasta la fecha, se ha dado por sentado que una dieta hiperproteica (HP) ocasionaba notables trastornos que exponemos, siguiendo a Barbany[2], en la figura 1.

El objetivo de la presente revisión es dar a conocer los resultados de los últimos estudios acerca de los efectos renales, metabólicos y óseos de las dietas HP, con el fin de tratar de esclarecer la controversia que, a día de hoy, el consumo de altas ingestas proteicas continúa generando.

A continuación, analizaremos con mayor detenimiento y a partir de estudios científicos relevantes, los efectos que la ingesta de una dieta HP pudieran provocar sobre los órganos y parámetros más susceptibles de ser alterados y el papel regulador que el ejercicio, y más concretamente por su relación con la síntesis proteica y/o la aplicación de carga, el entrenamiento de fuerza, pudiera tener.

Efectos metabólicos de una dieta hiperproteica

Tradicionalmente, las dietas HP se han asociado a una mayor ingesta de grasas. Esto es debido a que en la mayoría de las dietas occidentales, elevadas ingestas proteicas vienen asociadas a un mayor consumo de productos cárnicos, en las que los grasas animales son abundantes. Sin embargo, cuando dicha dieta HP es administrada de forma aislada, sin estar asociada a esas fuentes lipídicas, se ha demostrado que las dietas HP (ingestas superiores al 35% de proteína del total de la dieta), producen un descenso de la energía total ingerida, favorecen la pérdida de peso, reducen el acúmulo de grasa y mejoran el perfil lipídico plasmático general[12,13]. De hecho, tras varios meses consumiendo una dieta HP

no asociada a las mencionadas fuentes lipídicas tradicionales, los niveles de colesterol total, colesterol LDL y triglicéridos bajan[12,13], lo que puede significar una protección frente a enfermedades coronarias y renales[14-16].

Los suplementos proteicos basados en hidrolizados de lactosuero (proteína whey) en torno al 80-90% de riqueza, han ganado en popularidad en los últimos años, especialmente entre atletas y personas interesadas en ganancias de masa muscular[17]. Numerosos estudios desarrollados en humanos[18-21] y roedores[13,22,23] han demostrado la habilidad de dicha proteína para favorecer mejoras en la composición corporal (ayudando en el incremento de la masa muscular y reduciendo la deposición de grasa y las ganancias de peso). Sumado a esto, la proteína de lactosuero parece estar especialmente indicada para favorecer la pérdida de peso e incrementar la sensibilidad a la insulina[13,22].

Parte de los efectos beneficiosos de dichas dietas suelen ocasionarse como consecuencia de una reducción de la ingesta[22,24], y de ahí que haya una pérdida de peso al reducirse el aporte energético total de la dieta. Estas reducciones en el peso corporal han sido demostradas claramente en modelos animales[12,13,22,23].

Efectos metabólicos adicionales del ejercicio

Por otra parte, si dicha dieta se combina con ejercicio, especialmente de tipo aeróbico o de fuerza, vendrá asociada a menores niveles de colesterol y triglicéridos y una mejor composición corporal[16,25,26]. El entrenamiento de fuerza incrementa notablemente la masa, fuerza y potencia muscular[27], pero además, es una eficaz herramienta que reduce los niveles de grasa corporal, incrementa los niveles de colesterol HDL y disminuye los de colesterol LDL y triglicéridos, con la consecuente reducción de riesgo cardiovascular que ello conlleva[16,25,26].

Efectos renales de una dieta hiperproteica

Un consumo excesivo de proteína podría tener una un efecto renal adverso[28]. En particular, una ingesta excesiva de proteínas podría promover el daño renal al incrementar la presión glomerular y provocar una hiperfiltración renal[28]. Hay, sin embargo, cierta controversia al respecto en población sana. De hecho, algunos estudios sugieren que la hiperfiltración renal (el mecanismo propuesto como origen del daño renal) podría ser una respuesta adaptativa normal que acontece en respuestas a numerosas situaciones fisiológicas[29]. Hasta la fecha, sí se han comprobado los efectos beneficiosos de las restricciones proteicas sobre aquellas personas con insuficiencia renal o riesgo de formación de cálculos renales[30], sin embargo, en personas sanas, no se ha encontrado evidencia científica que demuestre un efecto adverso sobre la función renal[29].

La urea es el principal producto de desecho del metabolismo proteico en los mamíferos y el soluto más abundante en la orina. La excreción de urea es el resultado del proceso de filtración y de reabsorción pasiva a lo largo de la nefrona. El incremento de la concentración de urea plasmática y/o la ratio de filtración glomerular consecuencia del consumo de dietas HP se ha estudiado en modelos animales desde hace años[31]. Al ser necesario filtrar más urea, tiene que excretarse mayor cantidad de ella, lo que ocasionaría el mencionado estrés o sobrecarga renal.

En el reciente estudio de Frank et al[32], tras varios meses de dieta HP en hombres adultos sanos, se detectaron niveles plasmáticos elevados de urea, acido úrico, glucagón y niveles urinarios elevados de proteínas, albúmina y urea. Para estos autores, es necesario prestar mayor atención a los posibles efectos renales adversos que a largo plazo podría conllevar el mantenimiento de este perfil bioquímico plasmático y urinario[32]. Además, un exceso de proteína de origen animal (en principio más ácida por su contenido en sulfuros presentes en los aminoácidos) y más si cabe si se administra de forma conjunta con el desarrollo de ejercicio de alta intensidad (acidosis láctica), ocasionaría acidosis metabólica[33]. La acidosis metabólica intracelular estimula la hipocitraturia, que viene frecuentemente acompañada de hipercalcemia[33]. Tanto la hipocitraturia como la hipercalcemia urinarias contribuyen al riesgo de formación de cálculos renales de oxalato cálcico, principalmente a través del incremento en la saturación urinaria de sales de calcio[33,34]. La mayoría de los estudios[33-35] hablan de una reducción del citrato en torno a los 200-300 mg/día y un incremento del calcio urinario en torno a los 90-100 mg/día. Esta saturación urinaria de oxalatos de calcio se incrementa alrededor de un 35%, con lo que el balance se vuelve positivo, favoreciendo por tanto el riesgo de formación de cálculos renales. Grases et al (2006)[36], en un modelo experimental con ratas, observaron que la dieta hiperproteica facilita la nucleación heterogénea del ácido úrico y que con una dieta controlada, el efecto inhibitorio del citrato y el magnesio era más favorable respecto a dietas con exceso de lípidos, hidratos de carbono o proteínas.

Hammond y James[37] encontraron un incremento de entre el 26 y el 32% del peso de los riñones junto con un aumento notable de los mismos en ratas que consumieron una dieta HP durante dos semanas. Estos autores atribuyeron dicho incremento del peso y tamaño renal al fuerte efecto que los niveles elevados de proteína ocasionan sobre la producción de urea plasmática y la ratio de filtración diaria de nitrógeno.

Hasta que la evidencia científica sea más clara, y aunque esté probado que en personas sanas no existe riesgo renal, a nivel preventivo, los autores del presente manuscrito sugieren seguir las recomendaciones de Friedman[28]. Para dicho autor, debido a que la insuficiencia renal crónica es a menudo una enfermedad silenciosa, todos los individuos deberían analizar sus niveles plasmáticos de creatinina y realizarse una analítica de orina (con los valores casi momentáneos obtenidos en las tiras radiactivas para estimar si hay proteinuria urinaria sería suficiente), antes de iniciarse en el consumo de una dieta HP[28].

Efectos del ejercicio sobre la salud renal

El ejercicio físico ha demostrado actuar nuevamente como herramienta tamponadora de posibles daños fisiológicos, reduciendo la inflamación renal (disminuyendo el tamaño y peso del riñón) y mejorando los niveles de albúmina plasmática y la ratio de filtración glomerular[14,38].

Efectos óseos de una dieta hiperproteica

El consumo excesivo de proteínas también podría tener una afectación adversa sobre la salud ósea[39,40]. Partiendo de la teoría bioquímica lógica, el hueso ayudaría en la modulación del equilibrio ácido-base actuando como un sistema tamponador y regulador a través de la liberación de calcio[41,42]. Como ya se mencionaba anteriormente, el catabolismo de las proteínas genera amonio y libera sulfatos contenidos en los aminoácidos. El citrato y el carbonato cálcico del hueso son movilizados para neutralizar dichos ácidos, de ahí que, teóricamente, cuando aumentan las ingestas proteicas disminuya la densidad mineral ósea (como

consecuencia de la liberación de su principal mineral constituyente: el calcio) y la concentración urinaria de calcio se incremente[41,43], (con la consecuencia, ya mencionada en el apartado renal, del incremento del riesgo de formación de cálculos renales de oxalato cálcico[33,34]). Por lo tanto, dado que un consumo elevado de proteína de origen animal es acidogénico[39], promovería el fenómeno de resorción ósea[44]. Sin embargo, a pesar de que un exceso de proteína de alto poder acidogénico (ya sea proteína de origen animal o vegetal) podría afectar negativamente a la densidad mineral ósea, algunos estudios recientes han afirmado que este mencionado potencial acidogénico de la alta ingesta de proteínas y su consecuente impacto óseo podría ser compensado por otros nutrientes de la dieta (especialmente ciertos minerales presentes sobre todo en frutas y vegetales)[43,45,46]. De hecho, a pesar de que dicha resorción ósea sea posible, la evidencia científica es conflictiva[12,34,42,47-54]. Algunos estudios han mostrado efectos adversos de dietas HP en ratas[47,48] y humanos[49,50] mientras que otros, desarrollados en roedores sanos[12,34,42] y humanos de diferentes edades[51-53], no apreciaron una relación negativa, y dieron como resultado una baja ingesta proteica en detrimento de la densidad mineral ósea[51,54-56], con lo que se incrementa más si cabe la controversia generada al respecto.

Algunos autores defienden los efectos beneficiosos que sobre el metabolismo óseo puede tener una dieta HP cuando se consume junto a niveles apropiados de calcio, potasio y otros minerales, independientemente de la fuente de proteína consumida[41,43]. De hecho, los presentes autores destacan el estudio de Pye et al[57] desarrollado recientemente con ratas hembras, en el que se pretendían analizar los efectos del consumo elevado de proteínas a largo plazo, con y sin entrenamiento de fuerza. El consumo de dicha dieta HP (35% de riqueza), con el aporte suficiente de los mencionados minerales, redujo el peso y grasa de los animales, incrementó el peso libre de grasa y no ocasionó ningún efecto negativo sobre el hueso. Los autores concluyeron que una dieta al 35% de riqueza proteica, con contenido adecuado en calcio, puede ser beneficiosa a largo plazo para la salud ósea[57].

Por otra parte, Matsuo et al[58] reportaron mayor peso del fémur de las ratas a las que se les administró un *snack* deportivo hiperproteico, tanto si dicho *snack* era consumido tras un entrenamiento de fuerza como en grupos controles sedentarios.

Algunos investigadores han sugerido que las ingestas de calcio deben incrementarse cuando se incrementen los niveles de actividad física[59,60]. La excreción urinaria de calcio podría verse incrementada tras entrenamientos de alta intensidad y también podría producirse una pérdida de calcio a través del sudor[59,60]. Un estudio reportó que la excreción urinaria de calcio era un 70% superior en los periodos de entrenamiento comparados con los de recuperación o descanso[60], lo que podría estar relacionado con la acidosis metabólica ocasionada por el ejercicio anaeróbico.

Efectos del ejercicio sobre la salud ósea

Los beneficios del ejercicio, y más concretamente del entrenamiento de fuerza o del ejercicio que conlleve la aplicación de carga al hueso, sobre el contenido mineral óseo han sido altamente contrastados[60] tanto en animales[62-65] como en humanos[66-68]. El ejercicio parece tener mayor importancia que la dieta en relación con la densidad mineral ósea, principalmente por su efecto directo (a través de la carga)[69].

El hueso es un compartimento bastante estable, que cambia lentamente. Mayor número de investigaciones, especialmente diseñadas a largo plazo, son necesarias para esclarecer los efectos del consumo de altas dosis de proteína sobre la salud ósea. Es esta una cuestión que, tras décadas de estudio, sigue generando disparidad de opiniones en una sociedad en la que la osteoporosis se está convirtiendo en un problema sanitario cada vez más frecuente y costoso. De hecho, la fractura de cadera por pérdida mineral ósea es en nuestro país la primera causa de muerte accidental en mayores de 65 años y constituye el 75% de las muertes accidentales en mayores de 75 años[70]; a nivel mundial es una de las principales causas que derivan en hospitalización y fallecimiento en personas seniles[71,72].

A nuestro parecer, los efectos de las distintas combinaciones dieta-ejercicio sobre la salud ósea requieren de mayor estudio. El establecimiento de pautas concretas, tanto nutricionales como de prescripción de ejercicio físico, con verdadero efecto demostrable sobre el contenido mineral óseo y la calidad estructural del mismo, aún están por definir.

Hacia el establecimiento de nuevos niveles proteicos de referencia

A la vista de las evidencias científicas hasta la fecha, la Sociedad Internacional de Nutrición Deportiva (ISSN) concluyó en su documento de consenso, que cuando parte de un balance correcto de nutrientes, la ingesta de dietas HP no es perjudicial ni para la función renal ni para el metabolismo óseo de personas sanas y activas[5]. Además, en un estudio reciente se ha puesto en evidencia un importante aspecto que podría alterar las RDI proteicas que se establecían hasta la fecha. Elango et al[73] han demostrado que las recomendaciones de niveles proteicos mínimos y seguros de referencia de 0,66 y 0,8 g/kg/día respectivamente, de proteínas de alta calidad para adultos, estaban basadas en un metaanálisis de estudios del balance de nitrógeno que empleaban regresión lineal simple. Los mencionados autores reanalizaron dichos estudios del balance de nitrógeno que se emplearon usando análisis de regresión lineal multivariante y obtuvieron una media de niveles proteicos mínimos de referencia de entre 0,91 y 0,99 g/kg/día, respectivamente. Los valores medios de requerimientos seguros se establecieron entre 0,93 y 1,2 g/kg/día y son por lo tanto, entre un 41 y un 50%, respectivamente, superiores a las actuales RDI de proteínas de alta calidad en adultos[73]. Partiendo de estos nuevos rangos para la población sedentaria, en nuestra opinión, deben formularse nuevas recomendaciones de ingestas proteicas de referencia para atletas de las distintas disciplinas.

Debido a que la suplementación con aminoácidos no ha parecido mostrar un impacto positivo suficiente sobre el rendimiento deportivo, las recomendaciones acerca de su inclusión como norma han de ser conservadoras[74]. Desde un punto de vista práctico, es mucho más importante realizar un análisis nutricional completo del atleta, enfocado y adaptado a su disciplina deportiva y orientado a detectar carencias nutricionales, que recomendar suplementos proteicos sin una base objetiva lógica[74].

Conclusiones

Parece existir acuerdo acerca de los beneficios de las dietas HP sobre el perfil lipídico plasmático, que mejora los niveles de colesterol y triglicéridos y favorece la pérdida de peso. Sin embargo, los efectos de las dietas HP sobre parámetros renales y óseos aún desencadenan cierta controversia. Hay autores que no atribuyen riesgo renal alguno a la ingesta de dietas HP mientras que otros advierten del mayor riesgo de desarrollar una patología renal a largo plazo. Hasta que la evidencia científica sea

más clara, y aunque parece probado que en personas sanas no existe riesgo renal, en el campo de la prevención, debido a que la insuficiencia renal crónica es a menudo una enfermedad silenciosa, todos los individuos deberían analizar sus niveles plasmáticos de creatinina y detectar si existe proteinuria urinaria antes de iniciarse en el consumo de una dieta HP.

Respecto a los efectos de las altas ingestas proteicas sobre el metabolismo óseo, hay estudios que encuentran una menor densidad mineral ósea, otros que no encuentran diferencias significativas, y otros que atribuyen a las dietas HP un efecto protector óseo.

El hueso es un tejido que se altera muy lentamente (hacen falta años y no meses para detectar cambios) de ahí la dificultad de diseñar estudios en los que se analice este aspecto en humanos. Diseños experimentales con ratas que combinaran el ejercicio con altas ingestas proteicas durante periodos experimentales largos ayudarían a valorar y esclarecer el grado de afectación real y el papel regulador del entrenamiento de fuerza.

El ejercicio físico se presenta como una herramienta reguladora excelente ante la mayoría de las alteraciones que dichas dietas pudieran ocasionar, al fomentar un mejor perfil lipídico, reducir la inflamación renal, mejorar la ratio de filtración glomerular y estimular el fortalecimiento óseo.

Tras demostrarse en un reciente estudio que las ingestas proteicas diarias recomendadas de 0,8 g/kg/día estaban infravaloradas, y establecerse los nuevos niveles para población sedentaria, se hace necesario estimar los requerimientos proteicos apropiados para los atletas en sus diferentes disciplinas deportivas.

Mayor investigación sobre los efectos de la combinación de dichas dietas o suplementos deportivos con el ejercicio ayudarían a esclarecer la influencia real que dichas dietas, con y sin ejercicio, tienen sobre la salud renal y ósea.

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II

Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats

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Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats

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Abstract

Consumption of high-protein (HP) diets is postulated to exert a negative influence on bone and renal health. However, no conclusive evidence has been presented related to this issue or to the potential protective action of resistance training on HP-induced systemic effects. We examined the effects of HP diet consumption on food intake, body-weight gain, body composition, and renal, bone and metabolic parameters of rats performing resistance training. A total of ninety-six adult male Wistar rats were randomly distributed in twelve experimental groups (n 8): normal-protein (10 %) or HP (45 %) diets, with or without resistance training, killed for experimental periods of 1, 2 or 3 months. Diets were based on a commercial whey protein hydrolysate. Consumption of HP diets and resistance training significantly affected food intake, body weight and body composition, as well as the plasma levels of total cholesterol, HDL-cholesterol and TAG. The buffering action of resistance training on such diet-induced alterations was especially evident in the levels of plasma TAG. Consumption of HP diets led to 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 ($P<0{\cdot}05$). No apparent deleterious effect on bone mineral content was found. In conclusion, consumption of HP diets caused alterations in renal health status and some metabolic parameters, but did not seem to affect bone status. Resistance training had a protective action against alterations of renal health status and some metabolic parameters such as plasma TAG.

Key words: High-protein diets: Resistance training: Renal status: Bone status: Rats

The use of high-protein (HP) diets is a controversial topic. Resistance trainers receive confounding messages about the safety of purposely seeking ample dietary protein in their quest for enhanced protein synthesis, improved performance or maintaining health[1]. The current dietary reference intake for the general population is 0·8 g/kg body weight per d[2]. Individuals engaged in regular exercise training require more dietary protein than sedentary individuals[3]. Protein intake necessary to support N balance in strength athletes ranges from 1·2 to 1·7 g/kg body weight per d[3–5]. The International Society of Sports Nutrition position stand regarding protein and exercise[3] concluded that the concerns regarding the potential unhealthiness of protein intake within the range of 1·4–2·0 g/kg body weight per d are unfounded in healthy, exercising individuals, and were largely based on data from non-athletes[1]. However, some other studies have reiterated the tendency towards HP diet consumption by athletes and the potential health hazards posed by such diets[6].

HP diets appear to reduce energy intake, body-weight gain and fat deposition, and improve the plasma lipid profile[7–11]. Whey protein supplements, i.e. above 80 % protein concentrates or above 90 % protein isolates, have become popular among athletes and people interested in gaining muscle mass[12].

Excessive protein consumption might affect renal health[13,14]. Nevertheless, it is uncertain whether there is significant evidence to support this relationship in healthy individuals. In fact, some studies suggest that hyperfiltration, the purported mechanism for renal damage, is a normal adaptative mechanism that takes place in response

Abbreviations: CRM, certified reference material; HP, high protein; NP, normal protein; RM, repetition maximum.

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to several physiological conditions[15]. Recently, Jia *et al.*[13] have suggested that long-term intakes of protein at the upper limit of the acceptable macronutrient distribution range from whole protein sources may compromise renal health. On the other hand, Frank *et al.*[16] detected a significant increment in blood urea N, serum uric acid, glucagon, natriuresis, urinary albumin and urea excretion among healthy young men who consumed HP diets, and concluded that more attention should be paid to the potential adverse renal effects of such diets[16]. Moreover, relative excess of animal protein ingestion and strenuous physical exercise induce intracellular acidosis[17] that stimulates hypocitraturia, which is often accompanied by hypercalciuria[17]. Hypercalciuria and hypocitraturia contribute to the formation of Ca-containing kidney stones, mainly by increasing urinary saturation of Ca salts[17,18].

Excessive protein consumption may also affect bone health[19,20] due to its acidogenic effect that may result in bone resorption[21]. However, such acidogenic effect may be modified by other nutrients[22]. While it is possible that a prolonged HP intake may lead to increased bone resorption and ultimate bone loss, the current evidence on that issue is limited and conflicting. Some studies have reported a negative effect on bone health in both healthy rats[21,23] and human subjects[24,25]. Conversely, other HP studies in healthy rodents[7,18,26] and human subjects of different ages[27,28] did not show any relationship. In fact, it was reported that a low protein intake could have a negative effect on bone mineral density[29].

Resistance training can enhance absolute muscular strength, hypertrophy and muscular power[30], as well as reduce body fat, lipids and the consequent risk of CVD[31–33]. Furthermore, the benefits of exercise on bone health are highly contrasted by numerous studies[34]. However, in recent years, muscle dysmorphia has become an emerging condition that primarily affects male bodybuilders. Such individuals obsess about being inadequately muscular. Compulsions include spending hours in the gym, squandering excessive amounts of money on ineffectual sports supplements, abnormal eating patterns or even substance abuse[35,36].

Given the complexity of carrying out long-term interventional studies in human subjects and some ethical issues, animal experimental models are often used to study specific research questions. Bone is a complex tissue that changes slowly. As such, it is difficult to design and conduct well-controlled nutrition studies in human subjects to quantify the effect of one nutrient on bone[29]. The extrapolation of rodent studies to human subjects is widely found in the literature due to similar patterns of bone and renal structure and metabolism in both species[17,18,21,23,37]. Furthermore, the use of rodent experimental models is especially useful in bone metabolism, mainly because years, not weeks, are required to assess bone density change in human subjects[34]. The present study aimed: (1) to examine the effect of high-whey-protein consumption on body composition, lipid profile, and renal and bone parameters; (2) to examine the effect of resistance training on such outcomes and its potential interactions with a HP diet.

Experimental methods

Animals and experimental design

A total of ninety-six young albino male Wistar rats were allocated into four groups (n 24): normal-protein (NP)-sedentary group, NP-resistance training group, HP-sedentary group and HP-resistance training group. We splitted each group into three lots (n 8) that were killed 1, 2 and 3 months after the start of the experiment, thus resulting in twelve study groups.

The animals, with an initial body weight of 150 (SD 8) g, were housed from day 0 of the experiment in individual stainless steel metabolism cages designed for the separate collection of faeces and urine. The cages were located in a well-ventilated thermostatically controlled room (21 ± 2°C), with a relative humidity ranging from 40 to 60 %. A reverse 12 h light–12 h dark cycle (08.00–20.00 hours) was implemented to allow exercise training during the day. Throughout the experimental period, all rats had free access to double-distilled water and the animals consumed the two different diets *ad libitum*. One week before the experimental period, the rats were allowed to adapt to the diet and experimental conditions.

Body weight was measured weekly for all animals at the same time, and the amount of food consumed by each rat was registered daily.

On days 21, 49 and 77, a 12 h urine sample from each animal was collected for biochemical analysis. During these 12 h, located in the dark cycle, water was removed in order to avoid interferences with urine collection. Urine volumes were recorded, and samples were transferred into graduated centrifuge tubes for pH, Ca and citrate analysis.

At the end of the experimental period, the animals were anaesthetised with pentobarbital and killed by cannulation of the abdominal aorta. Blood was collected (with heparin as an anticoagulant) and centrifuged at 3000 rpm for 15 min to separate the plasma that was frozen in liquid N_2 and

Table 1. Composition of the experimental diets

Nutritional composition (g/100 g DM)	Normal-protein diet	High-protein diet
Whey protein	13·8	63·6
Mineral mix (AIN-93M-MX)	3·5	3·5
Vitamin mix (AIN-93-VX)	1	1
Fat (olive oil)	4	4
Choline chloride	0·25	0·25
Cellulose	5	5
Starch	58·8	20
Met	0·5	–
Sucrose	10	–

stored at −80°C. Carcass weight was recorded. Carcass is the weight of the slaughtered animal's cold body after being skinned, bled and eviscerated, and after removal of the head, the tail and the feet. Brown and white adipose tissue was extracted and weighed. Kidneys were extracted, weighed and immediately frozen in liquid N_2. Femurs, quadriceps and gastrocnemius were extracted, weighed and stored at −20°C.

All experiments were undertaken according to the Directional Guides Related to Animal Housing and Care (European Community Council, 1986), and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

Experimental diets

Formulation of the experimental diets is presented in Table 1. All diets were formulated to meet nutrient requirements of rats following the recommendations of the American Institute of Nutrition (AIN-93M)[38], with slight modifications. We selected a protein level of 45 % for the HP diet groups, following previous studies in which an HP diet was compared with NP diets in rats[7,8,11,18], whereas a protein content of 10 % was chosen for the NP diet groups. A commercial whey protein isolate was used as the sole source of protein since this protein source is widely available and used by sportsmen. Before diet preparation, total protein concentration of the commercial whey protein hydrolysate and its distribution among the protein or non-protein fractions were measured. Total N content of the commercial whey protein hydrolysate was 11·8 (SD 0·6) g/100 g DM, of which 11·7 % corresponded to insoluble N, whereas 85·4 % corresponded to soluble protein N and 2·9 % to soluble non-protein N.

Total protein concentration of the experimental diets was also assayed, with values of 44·3 (SD 2·1) % for the HP diet and 11·7 (SD 0·4) % for the NP diet (these values are adequate for our experimental design).

Chemical analyses

Moisture content was determined by drying to constant weight in an oven at 105 ± 1°C. Total N of the whey protein supplement was determined according to Kjeldahl's method. Crude protein was calculated as N × 6·25. Insoluble N and soluble protein and non-protein N were measured using the methodology described by Periago *et al.* (1996).

Bone and kidney ash were prepared by calcination at 500°C to a constant weight. Ca content was determined by atomic absorption spectrophotometry using a Perkin Elmer Analyst 300 spectrophotometer (Perkin Elmer, Wellesley, MA, USA). Analytical results were validated by standard references certified reference material (CRM)-189 (wholemeal starch; Community Bureau of Reference, Geel, Belgium), CRM-383 (haricot beans; Community Bureau of Reference) and CRM-709 (pig feed; Community Bureau of Reference). Mean values of five independent

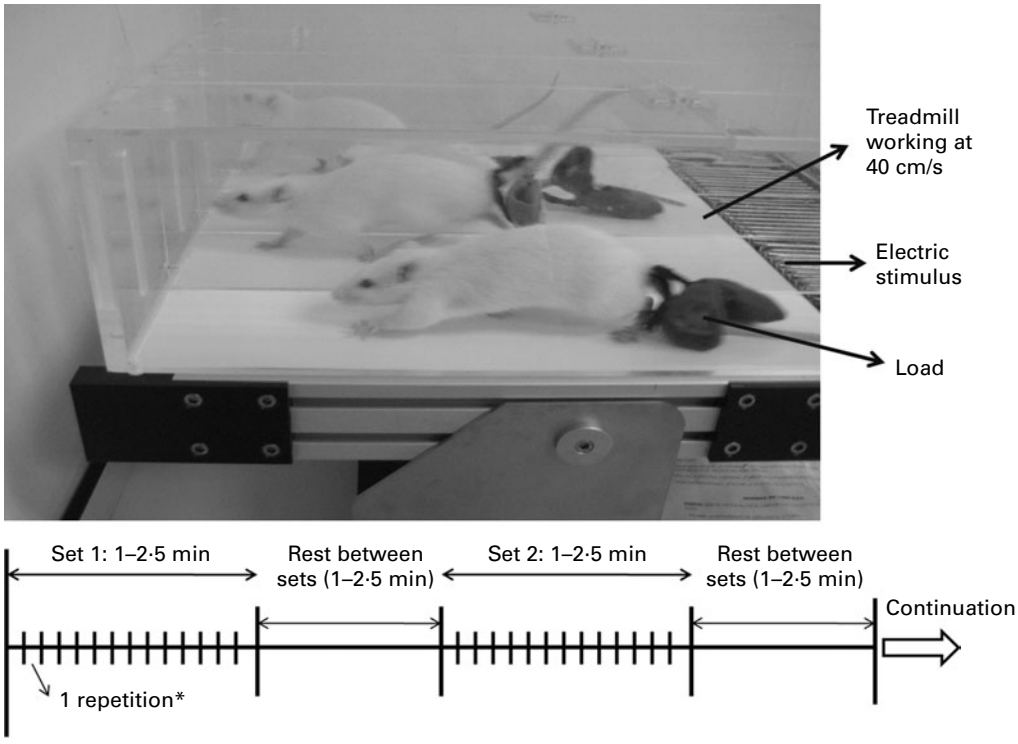

Fig. 1. Rats training on the treadmill and details of the resistance training protocol. Number of sets per training session: 10–12. Number of repetitions per set: 8–14. * A repetition is done when the resistance is transported along 20–40 cm or 2–4 s pulling the resistance although no distance is achieved.

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Table 2. Details of the resistance training programme

Weeks	Sessions/ week	Sets/ session	Set duration (min)	Rest between sets (min)	Load (% 1 RM)
1	4	10	2·0	1·0	55
2	3	10	2·0	1·0	60
3	4	10	2·0	1·0	65
4	3	10	2·0	1·5	70
5	4	10	2·0	1·5	70
6	3	10	2·5	1·5	75
7	4	12	2·5	1·5	75
8	3	12	2·0	2·0	80
9	4	12	2·5	2·0	80
10	3	12	1·5	2·0	85
11	4	12	2·0	2·5	85
12	3	12	1·0	2·5	90

RM, repetition maximum.

values for ash and Ca content were as follows: ash, CRM-383 = 2·48 (SEM 0·006) % *v.* certified value of 2·4 (uncertainty 0·1) %, CRM-709 = 4·29 (SEM 0·03) % *v.* certified value of 4·2 (uncertainty 0·4) %; Ca, CRM-383 = 2·78 (SEM 0·02) mg/g *v.* certified value of 2·9 (uncertainty 0·2) mg/g.

Urinary pH was analysed using a bench pH meter (Crison, Barcelona, Spain). Urinary citrate was measured using a commercial kit. Plasma urea, total cholesterol, TAG and HDL-cholesterol were measured using a HITACHI Roche p800 autoanalyser.

Resistance training

The experimental group was trained following a resistance protocol in a motorised treadmill (Panlab TREADMILLS for five rats, LE 8710R) with weights in a bag tied with a cord to the tail (Fig. 1). The training group exercised on alternate days (3–4 sessions/week). The animals ran at a constant speed of 40 cm/s during the whole experimental period (4, 8 or 12 weeks). Before exercise training, animals were adapted to the treadmill on a daily basis for 1 week, first 3 d without weight and the last 4 d with 20 % of their body weights. The number of sessions performed each week on alternate days, the number of sets per session and the time spent in each set as well as the load carried by the animals are shown in Table 2. From the first week of the experimental period until the completion of the study, the training weights (loads) were progressively increased and individually adjusted one time per week to the percentage of one repetition maximum (1 RM), defined as the maximum load that the rat could carry in the bag. The 1 RM test was conducted as follows: the rat was placed in a flat, horizontal and non-slippery surface with a specific loaded bag that was tied to its tail. The rat was acoustically stimulated and immediately reacted by moving forward. This procedure was repeated several times, increasing the load every time, until the load was so heavy that the rat could not move forward, yet actively stimulated. The load achieved at this point was considered the 1 RM and was weekly measured in all animals to adapt the %1 RM load during the training period. This type of training follows the established principles for human resistance training, involving weights, repetitions and sets to maximise gains in muscle strength[30]. All the training process was designed and controlled by sport scientists in collaboration with experienced researches used to work with rats. We considered that a repetition was finished when the rat stopped running and the next repetition began when the rat started running again, as a consequence of the electric stimulus at the end of the treadmill. The repetitions usually lasted 2–4 s, and the number of repetitions per set ranged between eight and fourteen repetitions (Fig. 1).

Animals in the control groups were managed identically to exercising animals, with the exception of exercise training. In order to avoid a possible confounding effect due to often handling in the training groups, control animals were handled weekly.

Statistical methods

Results are presented as means and standard deviations, unless otherwise indicated. The effects of dietary treatment and resistance training on the outcome variables were analysed by two-way repeated-measures ANOVA; with diet and exercise groups as fixed factors, and pre- and post-intervention values of body composition, lipid profile and renal and bone parameters as dependent variables. All analyses were performed separately for rats killed at 1, 2 and 3 months. All the analyses were performed using the Statistical Package for Social Sciences (SPSS, version 16.0 for Windows; SPSS, Inc., Chicago, IL, USA), and the level of significance was set at $P<0·05$.

Results

Food intake

Along the experimental period, food consumption gradually declined in all groups, especially from the second month. Food intake was higher in the NP groups when compared with the HP groups ($P<0·01$), and no significant differences were observed between the sedentary and exercise groups.

Body weight, body composition and lipid profile

The effects of NP diet *v.* HP diet and sedentary status *v.* resistance training on body weight, body composition and lipid profile are presented in Table 3. Final body weight was lower in the training and NP groups when compared with the sedentary and HP groups, respectively, particularly after 2–3 months of intervention ($P<0·01$). There was a significant interaction between diet and training ($P<0·05$), with a larger training-derived weight reduction in the NP groups than in the HP groups

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Table 3. Effect of normal-protein diet *v.* high-whey-protein diet and sedentary status *v.* resistance training on body weight, body composition and metabolic parameters in rats
(Mean values, standard deviations and percentages)

		Normal-protein diet					High-protein diet					*P*		
		Sedentary		Exercise			Sedentary		Exercise					
	Days	Mean	SD	Mean	SD	%*	Mean	SD	Mean	SD	%*	Diet effect	Training effect	Diet × training interaction
Final wt (g)	30	275	8·1	297	8·1	8·0	267	8·7	284	8·1	6·4	0·205	0·023	0·748
	60	317	11·5	281	11·4	−11·4	342	11·4	323	11·4	−5·6	0·007	<0·001	0·023
	90	339	11·0	314	11·0	−7·5	382	11·8	359	11·0	−6·0	<0·001	<0·001	0·041
Carcass (g)	30	131	3·4	124	3·4	−5·3	123	3·6	137	3·4	11·4	0·514	0·269	0·004
	60	141	4·7	139	4·7	−1·4	156	4·7	157	4·7	0·6	0·002	0·932	0·746
	90	154	5·8	153	5·8	−0·6	186	6·2	183	5·8	−1·6	<0·001	0·724	0·831
Brown fat (g)	30	0·96	0·07	0·87	0·07	−9·4	0·53	0·07	0·57	0·07	7·5	<0·001	0·718	0·332
	60	1·32	0·09	1·40	0·09	6·1	0·73	0·09	0·68	0·09	−6·8	<0·001	0·853	0·468
	90	0·83	0·09	0·88	0·09	6·0	0·84	0·10	0·61	0·09	−27·4	0·070	0·179	0·069
White fat (%)†	30	16·1	1·4	13·7	1·4	−14·9	11·9	1·5	11·4	1·4	−4·2	0·035	0·349	0·521
	60	22·3	2·0	11·7	2·0	−47·5	20·8	2·0	14·7	2·0	−29·3	0·693	0·000	0·265
	90	23·5	2·2	15·8	2·2	−32·8	14·7	2·3	14·1	2·2	−4·1	0·027	0·077	0·128
Quadriceps (mg/100 g animal wt)	30	725	0·3	677	0·3	−6·6	727	0·3	799	0·3	9·9	0·021	0·641	0·025
	60	659	0·3	767	0·3	16·4	733	0·3	863	0·3	17·7	0·013	0·001	0·728
	90	680	0·2	746	0·2	9·7	761	0·2	882	0·2	15·9	<0·001	<0·001	0·255
Gastrocnemius (mg/100 g animal wt)	30	647	0·2	638	0·2	−1·4	646	0·2	719	0·2	11·3	0·089	0·172	0·080
	60	603	0·3	745	0·3	23·5	658	0·3	763	0·3	16·0	0·179	0·000	0·501
	90	617	0·2	668	0·2	8·3	678	0·2	695	0·2	2·5	0·034	0·099	0·403
Total cholesterol (mg/l)	30	393	24	536	24	36·4	300	24	344	23	14·7	<0·001	0·001	0·048
	60	738	40	675	40	−8·5	406	40	325	40	−20·0	<0·001	<0·001	0·084
	90	479	32	507	32	5·8	479	31	356	30	−25·7	<0·001	0·024	0·148
TAG (mg/l)	30	479	46	443	46	−7·5	400	45	357	43	−10·8	0·078	0·384	0·929
	60	594	37	656	37	10·4	475	37	375	37	−21·1	<0·001	0·618	0·037
	90	536	46	488	43	−9·0	579	46	338	43	−41·6	0·237	0·003	0·039
HDL-cholesterol (mg/l)	30	285	23	489	23	71·6	90	23	241	21	167·8	<0·001	<0·001	0·245
	60	672	39	666	39	−0·9	305	39	207	39	−32·1	<0·001	0·195	0·252
	90	416	30	450	28	8·2	235	30	174	28	−26·0	<0·001	0·651	0·117

* Percentage of difference between the sedentary and exercise groups was computed as ((exercise − sedentary)/sedentary) × 100.
† Percentage of white fat related to carcass.

Table 4. Effect of normal-protein diet *v.* high-whey-protein diet and sedentary status *v.* resistance training on urea and kidney weight
(Mean values, standard deviations and percentages)

		Normal-protein diet					High-protein diet					*P*		
		Sedentary		Exercise			Sedentary		Exercise					
	Days	Mean	SD	Mean	SD	%*	Mean	SD	Mean	SD	%*	Diet effect	Training effect	Diet × training interaction
Urea (mg/l)	30	226	22	258	22	14·2	318	22	451	20	41·8	<0·001	0·001	0·027
	60	288	15	280	15	−2·8	324	15	356	15	9·9	0·001	0·442	0·200
	90	253	29	228	29	−9·9	598	31	376	29	−37·1	<0·001	<0·001	0·003
Kidney wet mass (g)	30	0·94	0·04	0·94	0·04	0·0	0·99	0·04	1·08	0·04	9·1	0·009	0·189	0·216
	60	0·94	0·05	0·97	0·05	3·2	1·21	0·05	1·15	0·05	−5·0	<0·001	0·651	0·319
	90	0·90	0·04	0·87	0·04	−3·3	1·42	0·04	1·21	0·04	−14·8	<0·001	0·005	0·040
Kidney wet mass (g/100 g carcass)	30	0·71	0·02	0·76	0·02	7·0	0·81	0·02	0·79	0·02	−2·5	0·004	0·539	0·156
	60	0·67	0·02	0·70	0·02	4·5	0·78	0·02	0·73	0·02	−6·4	0·006	0·667	0·084
	90	0·58	0·02	0·57	0·02	−1·7	0·76	0·02	0·66	0·02	−13·2	<0·001	0·019	0·092

* Percentage of difference between the sedentary and exercise groups was computed as ((exercise − sedentary)/sedentary) × 100.

(−11·4 *v.* −5·6% after the second month and −7·5 *v.* −6·0% after the third month). Carcass weight did not show differences between the sedentary and training groups, while it was heavier in the HP groups compared with the NP groups, particularly after the second and third month of the experimental period ($P<0·01$). White fat percentage (related to carcass weight) was lower in the HP groups when compared with the NP groups, especially after the third month ($P<0·05$), and in the training groups compared with the sedentary groups, with a larger effect on the second and third month.

Muscle weight (quadriceps and gastrocnemius) was generally higher for the HP groups and especially for the training groups, with a more stable and strong effect after the second month.

Plasma cholesterol levels were lower for the HP groups compared with the NP groups ($P<0·001$), and the training groups *v.* the sedentary groups ($P<0·01$ in the first 2 months and $P<0·05$ after the third month). There was a significant interaction between diet and training ($P<0·05$) on the levels of plasma TAG, with a larger training-derived reduction of plasma TAG in the HP groups when compared with the NP groups (−21·1 *v.* 10·4% after the second month and −41·6 *v.* −9·0% after the third month). HDL-cholesterol levels were considerably lower in the HP groups ($P<0·001$) when compared with the NP groups, whereas no differences were observed between the exercise and sedentary groups.

Urinary parameters and kidney weight

The effects of the NP diet *v.* the HP diet and sedentary status *v.* resistance training on plasma urea and kidney weight are expressed in Table 4. After 3 months of the

Table 5. Effect of normal-protein diet *v.* high-whey-protein diet and sedentary status *v.* resistance training on bone parameters
(Mean values, standard deviations and percentages)

		Normal-protein diet					High-protein diet					*P*		
		Sedentary		Exercise			Sedentary		Exercise					
	Days	Mean	SD	Mean	SD	%*	Mean	SD	Mean	SD	%*	Diet effect	Training effect	Diet × training interaction
Femur dry wt (g)	30	0·47	0·02	0·51	0·02	8·5	0·44	0·02	0·46	0·02	4·5	0·031	0·115	0·661
	60	0·54	0·02	0·54	0·02	0·0	0·55	0·02	0·53	0·02	−3·6	0·998	0·627	0·596
	90	0·57	0·02	0·58	0·02	1·8	0·62	0·02	0·64	0·02	3·2	0·021	0·514	0·656
Femur ash wt (g)	30	0·27	0·01	0·29	0·01	7·4	0·27	0·01	0·29	0·01	7·4	0·869	0·050	0·872
	60	0·34	0·01	0·34	0·01	0·0	0·35	0·01	0·34	0·01	−2·9	0·508	0·731	0·559
	90	0·36	0·01	0·37	0·02	2·8	0·40	0·01	0·42	0·01	5·0	0·009	0·321	0·683
Femur ash wt (mg/g dry femur)	30	578·4	5·3	572·7	5·6	−1·0	610·1	6·1	627·3	5·6	2·8	<0·001	0·323	0·055
	60	627·2	6·2	635·5	6·2	1·3	644·9	6·2	648·8	6·2	0·6	0·019	0·335	0·731
	90	632·4	4·4	646·9	4·8	2·3	643·4	4·8	649·6	4·5	1·0	0·152	0·034	0·380
Femur Ca content (mg/g dry femur)	30	195·4	6·6	177·2	5·6	−9·3	192·9	6·6	204·9	5·5	6·2	0·052	0·617	0·022
	60	193·5	9·0	192·4	9·0	−0·6	204·7	8·4	201·0	8·4	−1·8	0·268	0·783	0·881
	90	191·9	3·2	204·1	3·0	6·4	201·9	3·2	200·0	3·0	−0·9	0·359	0·115	0·034
Femur Ca content (mg/g ash)	30	335	11·6	311	9·8	−7·2	318	11·6	324	9·8	1·9	0·820	0·409	0·169
	60	310	13·8	304	13·8	−1·9	318	12·9	310	12·9	−2·5	0·623	0·602	0·938
	90	304	5·1	315	4·4	3·6	314	5·1	310	4·7	−1·3	0·592	0·479	0·111

* Percentage of difference between the sedentary and exercise groups was computed as ((exercise − sedentary)/sedentary) × 100.

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experimental period, there were significant diet and exercise effects on plasma urea, which was higher in the NP groups *v.* the HP groups and lower in the exercise groups *v.* the sedentary groups ($P<0·001$). Moreover, a diet × exercise interaction was found, with a higher reduction in urea plasma levels caused by training in the HP groups when compared with the NP groups (−37·6 *v.* −9·9 %, respectively) ($P<0·01$). The training effects on plasma urea were lower in both the HP and the NP experimental groups after the second and third month.

Kidney weight was higher in the HP groups when compared with the NP groups from the first month of the experimental period ($P<0·001$), whereas resistance training caused a significant reduction in the above-mentioned parameter after the third month of the experimental period. At that time, there was a significant diet × exercise interaction due to the more consistent effect of training on the HP group *v.* the NP group (−14·8 *v.* −3·3 % reduction, respectively). The same trend was observed when kidney wet mass values were expressed related to carcass weight.

Additionally (data not shown), after the second month of the experimental period, we analysed some urinary parameters, especially those related to metabolic acidity, such as urinary volume, urinary pH, urinary Ca and urinary citrate. Urine volumes were higher in the HP groups at all time points ($P<0·001$), without significant differences related to resistance training. Urinary pH was lower in rats that consumed the HP diets compared with those that consumed the NP diets ($P<0·001$). Urinary citrate was lower in the HP groups ($P<0·001$) when compared with the NP groups, whereas urinary Ca was significantly higher in the former groups ($P<0·01$). Regarding exercise effects, both urinary pH and Ca were slightly lower in the resistance training groups, although this effect was not statistically significant.

Bone parameters

The effects of the NP diet *v.* the HP diet and sedentary status *v.* resistance training on bone parameters are presented in Table 5. Femur dry weight, total ash and ash per g of DM were generally higher in the HP groups compared with the NP groups ($P<0·05$), and a similar effect was observed in the resistance training groups compared with the sedentary groups, although this effect was only significant for femur ash weight (mg/g dry femur) at the third month ($P<0·05$). No significant differences in femur Ca content related to diet or training were found.

Discussion

The main findings of the present study were (1) resistance training caused muscular hypertrophy and significantly reduced total cholesterol and TAG, with a more pronounced effect in the HP group. Resistance training was also effective at enhancing bone mineral content, as measured by femur ash weight (relative to dry femur weight). The effects of training were generally observed at the second and third month, suggesting a mid- to long-term effect. (2) HP diets showed a protective role on the bone mineral content and the lipid profile. (3) Some interactions between diet and training were found, such as the greater effects of resistance training on the lipid profile and kidney weight of rats that consumed the HP diet. (4) Serum and urinary markers showed metabolic acidity derived from high-whey-protein diet consumption; a fact that could explain the increased kidney weight observed in the HP groups. (5) Despite this higher urinary and plasma acidosis in the HP groups, bone was not affected.

Food consumption, body weight, body composition and lipid profile

In a similar way to what has been reported by other authors[7,8,11,39], food intake and body weight were altered by HP diet consumption. Body weight reduction may be attributed to a combined effect of the lower energy intake exhibited by the HP experimental groups, and the higher energy expenditure required by digestion and metabolism of protein foodstuffs. Alterations in body weight and food intake caused by HP diet consumption were probably on the basis of the lower plasma total- and HDL-cholesterol, and TAG exhibited by HP-fed animals. Furthermore, several human[9,10] and rodent studies[8,11] have demonstrated the ability of whey protein to improve body composition (increasing muscle mass and/or reducing body-weight gain and adiposity index). To note is that in the present study, the HP groups showed higher muscle mass (measured in quadriceps and gastrocnemius). Hypertrophy was also observed, as was expected, on the training groups, especially after 2 months of training.

Lower levels of total cholesterol and TAG could have a protective effect on cardiovascular and kidney disease[33,40,41]. Furthermore, we used whey protein, which appears to be especially indicated to avoid overweight and increased insulin sensitivity[8,11]. On the other hand, the exercise groups generally presented lower values of cholesterol and TAG, a fact that confirms the highly contrasted effects of resistance training exercise on body composition and lipid profile[31–33]. Especially noticeable is the interaction between diet and training on plasma TAG, a finding that points to a potentially more beneficial buffering effect of resistance training on plasma TAG of individuals who consume HP diets and could be at a higher risk of nutritional imbalance and renal alterations.

Renal parameters

Urea, the major end product of protein metabolism in mammals, is the most abundant solute in urine. Plasma

urea concentration and glomerular filtration rate increase when normal rats are fed HP diets[42]. More urea needs to be filtered, either because more of it has to be excreted, or because the efficiency of its excretion is reduced. This hyperfiltration might have deleterious consequences in diseased kidneys[42]. In fact, in the recent study of Jia *et al.*[13] performed in pigs, after 8 months of the experimental period, renal and glomerular volumes were 60–70 % higher for the HP group. These enlarged kidneys had greater evidence of histological damage, with 55 % more fibrosis and 30 % more glomerulosclerosis. Renal monocyte chemoattractant protein-1 and plasma homocysteine levels were also higher in the HP group. In the present study, kidneys from rats that consumed the HP diet were about 30 % higher. Furthermore, Hammond & Janes[43] found a 26–32 % increase in kidney fresh weight of rats after 2 weeks of HP diet consumption which they attributed to the strong effects on blood urea N and totally daily N filtration rate exerted by HP consumption.

On the other hand, in the study of Walrand *et al.*[44] performed in human subjects, younger subjects significantly increased their glomerular filtration rate following a HP diet, whereas older subjects did not show the same adaptation and showed a trend towards reduced glomerular filtration rate, thus clearly demonstrating a significant age-related difference in the response to HP intake.

In the present study, resistance exercise decreased kidney weight after 3 months of training, with a higher reduction in the HP experimental groups when compared with the NP experimental groups. A possible explanation could be that resistance training has been reported to reduce inflammation, increase serum albumin and increase glomerular filtration rate[40,45]. In the present study, a relatively short-term HP diet consumption (2 months) significantly increased plasma urea, urinary volume and urinary excretion of Ca, but at the same time, it decreased urinary pH and citrate. Since a decrease in urinary pH, hypocitraturia and hypercalcaemia are recognised risk factors for kidney stone formation[17,18], and such a tendency was observed in HP-fed rats under our experimental conditions, those animals could also be at risk of nephrolithiasis.

Bone parameters

The effects of HP diet consumption on bone health status appear to be a controversial issue, with authors either reporting a deleterious influence in healthy human subjects[24,25] and rats[21,22], or describing no adverse effects of HP supplements in rats[7,18,26,46] and human subjects[27,28]. In this regard, Heaney[47] have shown that HP diets have adverse effects on health only if dietary Ca and K intakes are not at the recommended levels. On the other hand, a negative relationship between low-protein diet consumption and bone mineral content has been reported[29,48,49]. Under the experimental conditions of the present study, it was interesting to find that the HP diet did not negatively affect bone health parameters such as ash content, but rather had a moderately protective effect on it.

As expected, urinary excretion of Ca was higher in the HP-fed experimental groups. Urinary Ca excretion is strongly related to net renal acid excretion[22,50]. The catabolism of dietary protein generates ammonium ion and sulphates from sulphur-containing amino acids. Bone citrate and carbonate are mobilised to neutralise these acids, so urinary Ca increases when dietary protein increases[22,50]. Protein is considered to be a net acid-producing substance and thus a net negative risk factor for bone dissolution. However, the acidogenic role of dietary protein remains a matter of debate, as it is well established that other food components have a clear impact on the acid–base balance[51,52].

There is substantial literature supporting the beneficial effects of HP consumption on skeletal metabolism when such protein is consumed together with adequate Ca, K and other minerals, regardless of the source of protein[22,50]. Pye *et al.*[37] found that lower body weight, fat mass and higher lean body mass were associated with a high-mixed-protein consumption by rats, but did not find any deleterious effect on bone. The authors concluded that a mixed HP diet containing adequate Ca levels and up to 35 % of energy can be deemed safe for long-term bone health.

Resistance training benefits on bone mineral content have been largely demonstrated[34]. In the present study, resistance training was also effective at enhancing bone mineral content, as measured by femur ash weight (relative to dry femur weight). These effects were observed after 3 months of intervention, suggesting a mid- to long-term effect. In the study of Bennell *et al.*[53] performed in rats, the authors did not appreciate differences in the bone of rats developing a similar training protocol. On the other hand, Burr *et al.*[54] reported that short periods of interrupted resistance training, with rest periods between them, were a more effective osteogenic stimulus than a single sustained session. Our training protocol meets effective characteristics mentioned by the authors.

The potential effects of HP diet consumption and strength training on bone health should be considered in relation to new and adjusted protein requirements. As a matter of fact, Elango *et al.*[55] have shown that dietary reference intake recommendations for mean and population-safe protein intakes of 0·66 and 0·8 g/kg per d, respectively, in adult human subjects are based on a meta-analysis of N balance studies using single linear regression analysis. The authors reanalysed existing N balance studies using two-phase linear regression analysis and obtained mean and safe protein requirements of 0·91 and 0·99 g/kg per d, respectively. The mean and population-safe requirements in adult men were determined to be 0·93 and 1·2 g/kg per d; requirements that are 41 and

50 % higher, respectively, than the current dietary reference intake recommendations[55].

Limitation and strengths

The present study has several limitations that need to be mentioned. First, the study lacked high technology instruments for the measurement of body composition and bone mineral density, such as dual-energy X-ray absorptiometry or the Universal Testing Machine for measuring femoral failure load. Second, the present physiological results obtained in rodents must be confirmed in human subjects. In other words, the effect seen over 2–3 months of a HP diet in rodents cannot be extrapolated directly to the potential effects over decades in human subjects. On the other hand, the present study involved an important number of rats allocated in different groups, so that the main effects of the HP diet, the main effect of resistance training and the interaction between them provide a good opportunity to comprehensively investigate how these lifestyle factors can influence some important health-related outcomes. In addition, some rats were killed after 1 month of intervention, others after the second month and others after the third month. These sequential killings allowed us to study whether diet and/or resistance training effects take place at a shorter or longer term.

In conclusion, high levels of whey protein consumption induced metabolic acidosis in rats, and seemed to negatively affect kidney, but not bone status. In fact, the HP diet showed a moderate positive effect on bone mineral content, as well as on other important health-related outcomes such as plasma lipid profile. The benefits of resistance training were clearly observed on several physiological parameters such as plasma total cholesterol, TAG and bone mineral content in both dietary protein levels studied. However, higher benefits were observed in the lipid profile and renal status of a more potentially compromised health situation such as that of experimental groups fed the HP diets.

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El entrenamiento de fuerza reduce la acidosis metabólica y la hipertrofia hepática consecuentes del consumo de una dieta hiperproteica en ratas

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Original

El entrenamiento de fuerza reduce la acidosis metabólica y la hipertrofia hepática y renal consecuentes del consumo de una dieta hiperproteica en ratas

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Resumen

Introducción: **El consumo de dietas hiperproteicas (HP) podría tener un efecto adverso sobre la acidosis metabólica y la salud hepática y renal. Sin embargo, existen pocos estudios que analicen los efectos del entrenamiento de fuerza sobre los parámetros sensibles de ser alterados por dichas dietas.**

Material y métodos: **Un total de 32 ratas Wistar adultas fueron distribuidas de forma aleatoria en 4 grupos experimentales (n = 8): dieta normoproteica o HP, con o sin entrenamiento de fuerza. Las dietas estuvieron basadas en un hidrolizado de proteína de lactosuero (whey). Tras 90 días de diseño experimental los animales fueron sacrificados para los posteriores análisis.**

Resultados y discusión: **El consumo de una dieta HP provocó acidosis metabólica (hipercalcemia e hipocitraturia urinarias, acidificación del pH urinario y niveles elevados de urea plasmática), (P < 0,05), e incremento del peso de hígado y riñón (P < 0,001). Así mismo, tras el consumo de dicha dieta HP, se redujeron los depósitos de tejido adiposo y los niveles plasmáticos de colesterol y triglicéridos (P < 0,05). El entrenamiento de fuerza mostró un efecto tamponador protector especialmente significativo en la reducción del peso del hígado, riñones, niveles de urea plasmática y triglicéridos plasmáticos y hepáticos (P < 0,001).**

Conclusiones: **El entrenamiento de fuerza redujo la acidosis metabólica y la hipertrofia hepática y renal ocasionada por la ingesta de una dieta HP en ratas a la vez que mejoró el perfil lipídico plasmático y hepático.**

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Palabras clave: *Dieta hiperproteica. Dieta normoproteica. Entrenamiento de fuerza. Acidosis metabólica. Perfil lipídico.*

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RESISTANCE TRAINING REDUCES THE METABOLIC ACIDOSIS AND HEPATIC AND RENAL HYPERTROPHY CAUSED BY THE CONSUMPTION OF A HIGH PROTEIN DIET IN RATS

Abstract

Introduction: **High protein (HP) diet consumption may adversely affect metabolic acidosis and hepatic and renal health. Despite such potentially adverse effect, there are only few studies analyzing the effects of resistance training on the parameters that could be altered by such diets.**

Material and methods: **A total of 32 adult male Wistar rats were randomly distributed in 4 experimental groups (n = 8): normoprotein or HP diets, with or without resistance training. Diets were based on a whey protein hydrolyzate, and the experimental period lasted for 90 days.**

Results and discussion: **Consumption of HP diets and resistance training significantly affected food intake, body composition and plasmatic levels of total cholesterol and triglycerides. Consumption of HP diets led to a considerable increase in liver and kidney weight (P < 0.001), urinary volume and acidity, as well as in the urinary excretion of Ca, with a parallel reduction in the urinary excretion of citrate (P < 0.05). The buffering action of resistance training on such diet-induced alterations was especially evident in the levels of hepatic and plasma triglycerides, plasmatic urea, and in liver and kidney weight (P < 0.001).**

Conclusion: **Resistance training had a protective action against alterations of hepatic and renal health status and some metabolic parameters like hepatic and plasma triglycerides.**

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Key words: *High protein diet. Normo-protein diet. Resistance training. Metabolic acidosis. Lipid profile.*

Abreviaturas

HP: Hiperproteica.
NP: Normoproteica.
LDL: Low Density Lipoprotein (lipoproteína de baja densidad).
HDL: High Density Lipoprotein (lipoproteína de alta densidad).
MCP-1: Renal monocyte chemoattractant protein-1.
N: Nitrógeno.
EEM: Error Estándar de la Media.
Ca: Calcio.
ANOVA: Análisis de la varianza.
MS: Materia Seca.

Introducción

Los posibles efectos adversos que una dieta hiperproteica (HP) pudieran ocasionar sobre la salud continúan sin ser completamente esclarecidos[1]. Las dietas HP producen un descenso de la energía total ingerida, favorecen la pérdida de peso, reducen el acúmulo de grasa y mejoran el perfil lipídico plasmático[2,3]. De hecho, tras varios meses consumiendo una dieta HP no asociada a altas fuentes lipídicas (como pueden ser los productos cárnicos), las concentraciones de colesterol total, colesterol LDL y triglicéridos se reducen[2,3].

Los suplementos proteicos basados en hidrolizados de lactosuero (también denominados suplementos de "proteína whey") han ganado en popularidad en los últimos años, especialmente entre personas interesadas en conseguir ganancias de masa y fuerza muscular[4]. Estudios desarrollados en humanos[5-8] y roedores[3,9-10] han demostrado la capacidad de dicha proteína para favorecer mejoras en la composición corporal, incrementando la masa muscular y reduciendo la deposición de grasa y el peso corporal. Sumado a esto, la proteína de lactosuero parece incrementar la sensibilidad a la insulina[9]. Parte de los efectos beneficiosos de dichas dietas suelen ocasionarse como consecuencia de una reducción de la ingesta[9,11], y de ahí que haya una pérdida de peso al reducirse el aporte energético total de la dieta.

Sin embargo, a pesar de estas virtudes, un exceso de proteína de origen animal, como es la de lactosuero, en principio más ácida por su mayor contenido en sulfuros contenidos en los aminoácidos, ocasionaría acidosis metabólica que podría comprometer a largo plazo la salud renal[12]. De hecho, un reciente estudio ha encontrado daño histológico e hipertrofia en riñones de cerdos que consumieron una dieta HP durante 8 meses[13].

En un estudio desarrollado en hombres adultos sanos, tras varios meses de consumo de una dieta HP, se detectó un aumento de la ratio de filtración glomerular, niveles plasmáticos elevados de urea, acido úrico y glucagón y niveles urinarios elevados de proteínas, albúmina y urea[14]. Sin embargo, a pesar de que el consumo a largo plazo de este tipo de dietas podría comprometer la salud renal, algunos autores sugieren que la hiperfiltración renal (el mecanismo propuesto como origen del daño renal) podría ser una respuesta adaptativa normal que acontece en respuestas a numerosas situaciones fisiológicas[15]. Hasta la fecha, se han comprobado los efectos beneficiosos de las restricciones proteicas sobre aquellas personas con insuficiencia renal o riesgo de formación de cálculos renales[16], pero, sin embargo, en personas sanas, no se ha encontrado evidencia científica que demuestre un efecto adverso de las dietas HP sobre la función renal, al menos a corto plazo[15,17].

El entrenamiento de fuerza incrementa notablemente la masa, fuerza y potencia muscular[20], pero además, es una herramienta eficaz para reducir los niveles de grasa corporal, incrementando los niveles de colesterol HDL y disminuyendo los de colesterol LDL y triglicéridos[21-23]. Sumado a esto, el entrenamiento de fuerza podría reducir la inflamación renal y mejorar los niveles plasmáticos de albúmina y la filtración glomerular[24-26].

Los estudios desarrollados en roedores han observado un notable incremento del peso del hígado tras el consumo de una dieta HP[18,19]. Por otra parte, el ejercicio aeróbico[27] y especialmente el de fuerza[28], parece disminuir los niveles de grasa en hígado en humanos. Además, el ejercicio físico ha demostrado reducir el estrés del retículo endoplasmático y la resistencia a la insulina en el tejido adiposo y hepático de ratas obesas[29]. Sumado a esto, en el estudio de Johnson et al.[27] el ejercicio aeróbico regular redujo los lípidos hepáticos en personas obesas, incluso sin la pérdida paralela de peso.

Debido a que pretendemos valorar los potenciales efectos adversos del consumo de una dieta HP, y a que son múltiples las variables que buscamos controlar de la forma más objetiva y cuantitativa posible, el presente estudio ha sido diseñado en ratas. Los objetivos del estudio fueron los siguientes: 1) Examinar los efectos del consumo de altas dosis de proteína sobre la composición corporal, el perfil lipídico, parámetros de acidosis metabólica, renales y hepáticos en ratas; 2) Valorar los efectos de un programa de entrenamiento de fuerza adaptado a la rata sobre dichas variables, y su posible interacción con la dieta.

Material y método

Animales y diseño experimental

Un total de 32 ratas Wistar macho albinas fueron distribuidas en 4 grupos experimentales: grupo de ratas con dieta normoproteica (NP) y sedentarias, grupo NP con entrenamiento de fuerza, grupo HP sedentarias y grupo HP con entrenamiento de fuerza (8 ratas por grupo).

Los animales, con un peso inicial de 150 ± 8 g, fueron alojados desde el primer día del periodo de adapta-

ción (una semana antes del comienzo del periodo experimental) en jaulas individuales de metabolismo diseñadas para la recogida por separado de heces y orina. Las jaulas estaban ubicadas en una habitación bien ventilada, termorregulada (21 ± 2º C), con un rango de humedad relativa de entre el 40 y el 60% y un ciclo inverso de luz de 12:12 horas (08:00-20:00 h). Durante todo el periodo experimental (90 días) los animales tuvieron pleno acceso al agua bidestilada, y consumieron los diferentes tipos de dietas *ad libitum*.

El peso de los animales fue registrado todas las semanas a la misma hora del día (08:00 de la mañana), tras 12 horas de ayunas. Así mismo, la cantidad de dieta diaria consumida por cada unos de los animales fue cuantificada.

A los 89 días del periodo experimental, una muestra de 12 horas de orina de cada animal fue recogida para su análisis bioquímico. Durante esas 12 horas, localizadas en el ciclo de oscuridad para hacerlas coincidir con el periodo previo al pesaje de los animales, el agua y la comida fueron retirados para evitar interferencias en la recogida debidas a que los animales suelen verter agua bidestilada y comida sobre la orina, lo que altera los marcadores urinarios. La orina fue posteriormente pesada y transferida a tubos graduados para el análisis del pH, volumen, calcio y citrato urinarios.

Al final del periodo experimental los animales fueron anestesiados con pentobarbital y desangrados mediante canulación de la arteria aorta abdominal. La sangre fue extraída (con heparina como anticoagulante) y centrifugada a 3.000 rpm durante 15 minutos para separar el plasma, que fue congelado en Nitrógeno (N) líquido y conservado a -80º. La grasa parda y blanca fue extraída y pesada. Los riñones y el hígado fueron extraídos, pesados y congelados inmediatamente en N líquido. Para analizar el efecto de las distintas intervenciones sobre la grasa y la masa muscular, que pudieran verse afectadas por el entrenamiento (posibles ganancias de masa muscular y pérdida de masa grasa) o la dieta (pérdida de grasa debida a la dieta HP), se cuantificó el peso de la carcasa del animal. La carcasa corresponde al peso del animal tras ser sacrificado y desangrado, sin piel, grasa, cabeza, extremidades ni vísceras.

Todos los experimentos fueron desarrollados de acuerdo a la Guía Europea de Alojamiento y Cuidado Animal (European Community Council, 1986)[30] y todos los procedimientos que se han llevado a cabo en el presente estudio han sido aprobados por el Comité Ético de Experimentación Animal de la Universidad de Granada.

Determinaciones analíticas

Previamente al análisis de las dietas y el hidrolizado proteico empleado en las mismas, toda la materia fue llevada a peso constante en un horno a 105 ± 18º C. El contenido en N total del suplemento de hidrolizado proteico de lactosuero fue determinado mediante el método de Kjeldahl. El contenido en proteína cruda fue calculado como N x 6,25. El contenido de N insoluble y el de N soluble proteico y no proteico fueron analizados empleando la metodología descrita por Periago et al.[31]

El contenido de Ca urinario fue determinado, tras la dilución de las muestras, por espectrofotometría de absorción atómica (Perkin Elmer Analyst 300, PerkinElmer, Wellesley, MA, USA). Se llevó a cabo una calibración externa, evaluando la exactitud del método mediante el análisis de un material de referencia certificado de músculo bovino (NIST CRM 8414 bovine muscle powder, Gaithersburg MD, USA). Los valores medios y errores estándar de la media (EEM) de cinco mediciones independientes para cenizas y contenido en Ca fueron los siguientes: Cenizas, CRM-383 = 2,48 ± 0,006% *vs* valor certificado de 2,4 ± 0,1% de variación, CRM-709 = 4,29 ± 0,03% *vs* valor certificado de 4,2 ± 0,4% de variación; Ca, CRM-383 = 2,78 ± 0,02 mg/g vs. valor certificado de 2,9 ± 0,2 mg/g de variación).

El citrato urinario se valoró empleando un kit comercial (Boehringer Mannheim, Cat. Nº. 10 139 076 035), y el pH urinario usando un analizador de pH (Crison, Barcelona, España).

La extracción de la grasa del hígado se llevó a cabo mediante el método de Folch con ligeras adaptaciones[32]. Para la determinación del colesterol y triglicéridos hepáticos se utilizaron kits comerciales (Spinreact, S.A. Gerona, España).

La urea, bilirrubina, colesterol total y niveles de triglicéridos en plasma fueron analizados con un autoanalizador (HITACHI, Roche p800).

Dietas experimentales

La formulación de las dietas experimentales se muestra en la tabla I. Para cumplir con los requerimientos nutricionales de la rata, todas las dietas fueron formuladas siguiendo las recomendaciones del Instituto Americano de Nutrición (AIN-93M)[33]. El porcentaje de riqueza proteica para las dietas HP se estableció en un

Tabla I
Composición de las dietas experimentales

Composición nutricional (g/100 g MS)	*Dieta normoproteica (10%)*	*Dieta hiperproteica (45%)*
Hidrolizado proteico de lactosuero (72,5% de riqueza)	13,8	63,6
Complemento mineral (AIN-93M-MX)	3,5	3,5
Complemento vitamínico (AIN-93-VX)	1	1
Grasa (aceite de oliva)	4	4
Colina	0,25	0,25
Celulosa	5	5
Almidón	58,8	20
Metionina	0,5	–
Sacarosa	10	–

MS: Materia seca.

45% dado que es el valor medio empleado para dietas HP en diseños experimentales similares desarrollados en ratas[2-3,9,34]. Del mismo modo, la dieta NP se estableció en un 10% de riqueza. Con el fin de reproducir de la forma más real posible los hábitos nutricionales de deportistas y aficionados, se ha empleado un hidrolizado deportivo comercial de lactosuero como fuente proteica (Dymatize ISO-100, Farmers Branch, TX, USA). Previo a la elaboración de las dietas, la concentración proteica del hidrolizado comercial y su distribución (fracciones de N proteicas y no proteicas) fueron analizadas. El contenido total de N del hidrolizado comercial proteico de lactosuero fue de 11,8 ± 0,6 g/100 g de sustancia seca, lo que corresponde a un 72,5% de riqueza total. El contenido en N de dicho hidrolizado estuvo distribuido de la siguiente forma: 11,7% correspondió a N insoluble, 85,4% a N soluble proteico y el 2,9% restante a N soluble no proteico.

Una vez elaborada la dieta con sus distintos componentes, la concentración de proteína tanto para las dieta HP como NP fue analizada, obteniéndose unos valores de 44,3 ± 2,4% y 11,7 ± 0,4%, respectivamente (estos niveles son adecuados para nuestro diseño experimental). Debido a la alta calidad de la proteína (alto valor biológico) del suplemento deportivo proteico empleado (proteína de lactosuero), no hemos considerado necesaria la complementación de la dieta HP con otros aminoácidos, sin embargo, en las dietas normoproteicas hemos añadido 0,5 gramos/100 g de dieta de metionina[33].

Entrenamiento de fuerza

Los grupos experimentales de ejercicio llevaron a cabo un protocolo de entrenamiento de fuerza en un tapiz rodante motorizado de diseño especial para ratas (Panlab TREADMILLS con 5 calles para el entrenamiento simultáneo de 5 ratas, LE 8710R) con cargas en un saco atado a la cola. El entrenamiento se desarrolló en días alternos, a una velocidad constante de 40 cm/s durante todo el periodo experimental (12 semanas). Previamente al periodo experimental, los animales fueron adaptados al tapiz rodante mediante una sesión diaria durante una semana de adaptación en la que los primeros 3 días corrían sin carga y los 4 últimos arrastrando el 20% de su peso corporal. El protocolo de entrenamiento de fuerza empleado ha sido previamente descrito por Aparicio et al.[35]

Los animales sedentarios fueron manipulados de forma exacta a los grupos de entrenamiento, con la excepción del propio entrenamiento, con el fin de evitar un efecto contaminante debido al contacto humano.

Análisis estadístico

Los resultados se presentan como media y desviación estándar de la media a no ser que se indique lo contrario. Los efectos de la dieta y el entrenamiento de fuerza sobre las distintas variables incluidas en el estudio fueron analizados mediante un análisis de la varianza (ANOVA) de medidas repetidas, donde el nivel proteico y el entrenamiento de fuerza eran los tratamientos empleados. Todos los análisis se llevaron a cabo con el software estadístico SPSS, versión 16.0 para Windows (SPSS Inc., Chicago, IL), y el nivel de significación se estableció en 0,05.

Resultados

Ingestas, peso, composición corporal y perfil lipídico

Las diferencias en ingestas, peso, composición corporal y el perfil lipídico entre grupos de animales siguiendo una dieta HP o NP, sedentarios o con entrenamiento de fuerza, se muestran en la tabla II. La

Tabla II
Efectos de una dieta normoproteica vs hiperproteica en ratas sedentarias o sometidas a entrenamiento de fuerza sobre el peso, composición corporal y perfil lipídico

	Dieta normoproteica			*Dieta hiperproteica*			*P*		
	Sedentario	*Ejercicio*	*%**	*Sedentario*	*Ejercicio*	*%**	*Efecto de la dieta*	*Efecto del entrenamiento*	*Interacción dieta × entrenamiento*
Ingesta diaria (g)	17,8 ± 0,3	15,7 ± 0,4	-11,8	15,8 ± 0,3	14,5 ± 0,4	-8,2	< 0,001	< 0,001	0,308
Peso final (g)	339 ± 11,0	314 ± 11,0	-7,5	382 ± 11,8	359 ± 11,0	-6,0	< 0,001	< 0,001	0,041
Carcasa (g)	154 ± 5,8	153 ± 5,8	-0,6	186 ± 6,2	183 ± 5,8	-1,6	< 0,001	0,724	0,831
Grasa parda (g)	0,83 ± 0,09	0,88 ± 0,09	6,0	0,84 ± 0,10	0,61 ± 0,09	-27,4	0,070	0,179	0,069
Grasa blanca (%)[#]	23,5 ± 2,2	15,8 ± 2,2	-32,8	14,7 ± 2,3	14,1 ± 2,2	-4,1	0,027	0,000	0,128
Colesterol total (mg/dl)	47,9 ± 3,2	50,7 ± 3,2	5,8	47,9 ± 3,1	35,6 ± 3,0	-25,7	< 0,001	0,024	0,148
Triglicéridos (mg/dl)	53,6 ± 4,6	48,8 ± 4,3	-9,0	57,9 ± 4,6	33,8 ± 4,3	-41,6	0,237	0,003	0,039

Valores expresados como media ± desviación estándar. [#]Porcentaje de grasa blanca con respecto al peso de la carcasa. *El porcentaje de diferencia entre grupo sedentario y con ejercicio fue calculado como [(ejercicio-sedentario)/sedentario] x 100.

Tabla III
Efectos de una dieta normoproteica vs hiperproteica en ratas sedentarias o sometidas a entrenamiento de fuerza sobre variables de acidosis metabólica y hepáticas

	Dieta normoproteica			*Dieta hiperproteica*			*P*		
	Sedentario	*Ejercicio*	*%**	*Sedentario*	*Ejercicio*	*%**	*Efecto de la dieta*	*Efecto del entrenamiento*	*Interacción dieta × entrenamiento*
Peso riñón (g)	0,90 ± 0,04	0,87 ± 0,04	-3,3	1,42 ± 0,04	1,21 ± 0,04	-14,8	< 0,001	0,005	0,040
Urea plasmática (mg/dl)	25,3 ± 2,9	22,8 ± 2,9	-9,9	59,8 ± 3,1	37,6 ± 2,9	-37,1	< 0,001	< 0,001	0,003
pH urinario	6,67 ± 0,08	6,34 ± 0,08	-4,9	6,08 ± 0,08	5,79 ± 0,08	-4,8	< 0,001	0,001	0,826
Volumen Urinario (ml)	3,00 ± 0,3	2,10 ± 0,3	-30,0	4,44 ± 0,4	3,34 ± 0,3	-24,8	0,001	0,007	0,772
Calcio Urinario (mg/dl)	2,27 ± 0,7	1,71 ± 0,7	-24,7	10,90 ± 0,7	4,40 ± 0,7	-59,6	< 0,001	< 0,001	< 0,001
Citrato Urinario (mg/dl)	3,71 ± 0,5	5,40 ± 0,5	45,6	2,41 ± 0,5	1,20 ± 0,5	-50,2	< 0,001	0,656	0,007
Peso hígado (g)	7,66 ± 0,4	7,05 ± 0,4	-7,8	12,87 ± 0,4	9,03 ± 0,4	-30,2	< 0,001	< 0,001	< 0,001
Grasa hígado (mg)	7,26 ± 0,9	6,85 ± 0,9	-5,6	5,21 ± 0,9	5,41 ± 0,9	3,8	0,075	0,911	0,745
Colesterol total hígado (mg)	4,72 ± 0,6	3,49 ± 0,6	-26,1	5,37 ± 0,6	5,29 ± 0,5	-1,5	0,037	0,248	0,305
Triglicéridos hígado (mg)	5,35 ± 0,5	2,00 ± 0,4	-62,6	1,27 ± 0,4	1,79 ± 0,4	40,9	< 0,001	0,002	< 0,001
Bilirrubina plasmática (mg/dl)	0,075 ± 0,02	0,023 ± 0,2	-69,3	0,037 ± 0,02	0,052 ± 0,024	40,5	0,820	0,389	0,131

Valores expresados como media ± desviación estándar. *El porcentaje de diferencia entre grupo sedentario y con ejercicio fue calculado como [(ejercicio-sedentario)/sedentario] x 100.

ingesta diaria media fue menor en los grupos HP con respecto a los NP y en los de entrenamiento comparados con los sedentarios (ambas P < 0,001). El peso final de los animales fue menor para los grupos entrenamiento, especialmente en el grupo NP (P < 0,01), existiendo una interacción dieta × entrenamiento (P < 0,05), con una mayor reducción de peso como consecuencia del entrenamiento en los grupos NP con respecto a los HP (-7,5 *vs* -6,0%). El porcentaje de grasa blanca fue inferior en los grupos HP comparado con los NP (P < 0,05).

Los niveles de colesterol plasmáticos fueron inferiores para los grupos HP en comparación con los NP (P < 0,001) y en los grupos de ejercicio comparado con los sedentarios (P < 0,05). Se ha presentado una interacción dieta × entrenamiento en los niveles de triglicéridos (P < 0,05), con un efecto mayor de reducción en el grupo HP que en el NP (-41,6 *vs* -9,0%).

Parámetros urinarios, renales, de acidosis metabólica y hepáticos

Los efectos de la dieta y del entrenamiento sobre los niveles de urea, peso de hígado y riñón, parámetros urinarios de acidosis metabólica y perfil lipídico hepático para grupos NP e HP, con y sin entrenamiento de fuerza, se muestran en la tabla III. Tras 90 días de periodo experimental, el peso del riñón del grupo HP sedentario fue un 58% superior con respecto al NP y un 33% superior en el HP con entrenamiento (P < 0,001). Los niveles de urea plasmática fueron mayores en los grupos HP comparados con los NP y menores en los grupos de entrenamiento en comparación con los sedentarios (P < 0,001). Además, se ha presentado una interacción dieta × entrenamiento, existiendo una mayor reducción de los niveles de urea en los grupos donde el entrenamiento se combinaba con la dieta HP que con la NP (-37,6% *vs* -9,9%, respectivamente) (P < 0,01).

La diuresis fue mayor en los grupos HP (P < 0,001), sin diferencias atendiendo al efecto del ejercicio. El pH urinario fue menor (más ácido), para las ratas que consumieron dietas HP (P < 0,001). El citrato urinario fue inferior en los grupos HP (P < 0,001) con respecto a los NP y el Ca urinario superior (P < 0,01). En relación con el ejercicio, tanto el pH como el Ca urinario fueron ligeramente inferiores en los grupos de entrenamiento, aunque sin significación estadística.

El peso de hígado fue notablemente inferior para los grupos NP con respecto a los HP (P < 0,001) y para los grupo de entrenamiento con respecto a los sedentarios (P < 0,001). Además, se presentó una interacción dieta × entrenamiento, siendo mayor la reducción del peso del hígado en la dieta HP que en la NP (-7,8% en la NP *vs* -30,2% en la HP). La grasa del hígado fue menor en el grupo que consumió dieta HP pero sin llegar a la significación (P = 0,07). Las concentraciones de colesterol hepático fueron superiores para los grupos de dieta HP (P < 0,05). La concentración de triglicéridos hepáticos fue menor en los grupos HP (P < 0,001) y éstos fueron un 62% más bajos en el grupo NP con entrenamiento (P < 0,01), con interacción dieta × entrenamiento (P < 0,001). Por último, no se han encontrado diferencias significativas en los niveles plasmáticos de bilirrubina.

Parte de los resultados que se presentan en el presente artículo (variables descriptivas de peso, composición corporal y perfil lipídico plasmático de la tabla II y peso de riñón y niveles de urea plasmática de la tabla III) provienen de un amplio estudio del que parte ha

sido publicado [35], pero que a juicio de los autores, deben ser incluidos al ser variables descriptivas básicas para dar sentido a la globalidad del análisis que se ha llevado a cabo.

Discusión

Altas ingestas de proteína indujeron un estado de acidosis metabólica en ratas, lo que podría afectar negativamente al hígado y riñón, que presentaron hipertrofia. Por otra parte, las dietas HP mostraron un efecto positivo sobre el perfil lipídico, lo cual podría tener un efecto protector cardiovascular, renal y hepático. Los beneficios del entrenamiento de fuerza fueron generalizados, mejorando la composición corporal y el perfil lipídico y atenuando la hipertrofia de hígado y riñones y la excreción de urea plasmática, derivados del consumo de dietas HP. Hasta la fecha, no existen estudios donde la interacción dieta NP e HP y entrenamiento de fuerza haya sido analizada. Mediante el diseño experimental y análisis estadístico del presente artículo podemos comprobar qué efectos son provocados por la dieta y cuáles por el entrenamiento de fuerza, de forma tanto aislada como conjunta.

Tal y como ya ha sido descrito por otros autores, la ingesta fue menor en los grupos de dieta HP[2-3], lo que pudo favorecer unas menores concentraciones de colesterol total y triglicéridos que podrían tener un efecto protector sobre enfermedades cardiovasculares, hepáticas y renales[23,25,36]. Este fenómeno puede generar cierta controversia, dado que a priori, dichas dietas HP podrían mejorar el perfil lipídico, pero a costa de un riesgo paralelo sobre el equilibrio nutricional, acidosis metabólica, alteraciones renales y hepáticas. Por otra parte, los grupos entrenamiento presentaron menor contenido de grasa corporal y mejor perfil lipídico general, aspecto que confirma los efectos metabólicos altamente contrastados del entrenamiento de fuerza sobre dichos parámetros[21-23]. Tal y como ha sido descrito anteriormente por nuestro grupo de investigación[35], es especialmente reseñable la interacción observada entre dieta-ejercicio y niveles plasmáticos de triglicéridos, donde el entrenamiento de fuerza se muestra especialmente eficaz reduciendo dichas concentraciones plasmáticas cuando se combina con el consumo de dichas dietas HP.

La concentración plasmática de urea aumentó con el consumo de la dieta HP tal y como ha sido observado por otros autores[37]. Al ser necesario filtrar mayor urea, más urea es excretada, lo que ocasiona una hiperfiltración que podría generar problemas renales[13-14,37]. Los efectos hemodinámicos de la dieta HP han sido atribuidos a mecanismos como el incremento de la velocidad de filtración glomerular y el incremento de la proteinuria, que pueden derivar en glomerulosclerosis e insuficiencia renal. Los responsables de estos mecanismos pueden ser las hormonas (glucagón, insulina, somatomedina C y angiotensina II), las citoquinas (prostaglandinas) y las quininas. También puede estar implicada la regulación renal de los transportadores de sodio, ya que se ha observado que la actividad de estos transportadores está aumentada en respuesta al aumento de la filtración de aminoácidos, estimulando la retroalimentación túbulo-glomerular y aumentando la filtración glomerular[38]. Los riñones de los animales que consumieron una dieta HP presentaron un peso final en torno a un 45% superior. Hammond y James[19] también encontraron un incremento del 30% del peso del riñón tras tan solo 2 semanas de consumo de una dieta HP del 46% de riqueza. Estos autores atribuyeron dicha hipertrofia renal al fuerte impacto metabólico de los altos niveles de urea plasmática observados y a la forzada mayor filtración de N ocasionada por el consumo de las dietas HP.

De hecho, a pesar de la evidencia acerca del potencial efecto anti-obesidad de este tipo de dietas, el impacto renal de altos consumos de proteína sigue sin esclarecerse[12-16]. En un reciente estudio desarrollado en cerdos (por la similitud de los riñones de estos animales con los de los humanos), tras 8 meses de periodo experimental, el volumen renal y glomerular fue entre un 60-70% superior en el grupo de dieta HP. Estos mayores riñones también presentaron daño histológico, con un 55% más de fibrosis y un 30% más de glomerulosis. Además los niveles plasmáticos de homocisteína y de MCP-1 (renal monocyte chemoattractant protein-1) fueron muy superiores (la MCP-1 es una quimiocina que es marcador de inflamación renal)[13].

En nuestro estudio, tras un periodo de tiempo relativamente corto (3 meses) de exposición a una dieta HP, se produjo un incremento significativo de los niveles plasmáticos de urea, del volumen urinario y de la excreción urinaria de Ca (hipercalciuria), a la par que se produjo un descenso del pH urinario (más ácido) y una menor excreción urinaria de citrato (hipocitraturia). Tanto la hipercalciuria como la hipocitraturia son reconocidos factores de riesgo para la formación de cálculos renales de oxalato cálcico[12,34], por lo que los animales alimentados con dietas HP presentarían, en principio, un mayor riesgo de padecer nefrolitiasis.

El entrenamiento de fuerza logró paliar en parte la hipertrofia renal ocasionada por la dieta HP. Entre los mecanismos que pudieran explicar este efecto protector renal del ejercicio se encuentra el hecho de que el entrenamiento de fuerza parece reducir la inflamación renal, aumentar la velocidad y fracción de filtrado glomerular y las concentraciones de albúmina plasmática[24-26]. De hecho, en el reciente estudio de Pinheiro-Mulder et al.[26] han concluido que el ejercicio podría ser una eficaz práctica auxiliar enfocada a atenuar las alteraciones renales que se observan en personas que presentan obesidad inducida por la dieta.

El incremento del peso del hígado tras el consumo de la dieta HP fue porcentualmente ligeramente superior al del riñón, con un 68% mayor peso fresco para el grupo sedentario de dieta HP. Sin embargo, para el

grupo HP con entrenamiento el incremento fue tan solo un 28% superior, consecuentemente podemos extraer una de las interacciones dieta-ejercicio más notables del presente estudio: el entrenamiento de fuerza es especialmente eficaz reduciendo la hipertrofia hepática inducida por la dieta HP.

Así como numerosos estudios han analizados los efectos hepáticos de dietas altas en grasas, son casi inexistentes aquellos que hayan analizados los efectos de dietas HP sobre dicho órgano[19]. En el anteriormente mencionado estudio de Hammond y Janes[19] también se analizó el peso del hígado en sustancia fresca y, al igual que en nuestro estudio, detectaron un incremento del peso en sustancia fresca de en torno al 20%. Este menor incremento, con respecto al observado en el presente estudio, puede ser debido a que la exposición a la dieta HP fue de tan sólo 2 semanas, frente a los 3 meses de nuestro diseño experimental, sumado a que es posible que la hipertrofia hepática se presente a más largo plazo. Por tanto, se hacen necesarios mayor número de estudios, especialmente histológicos, que analicen el posible efecto adverso sobre la salud hepática que a largo plazo podrían tener este tipo de dietas.

El hígado humano contiene proporcionalmente más ácidos grasos saturados y poli-insaturados que el tejido adiposo subcutáneo e intra-abdominal[39]. Se ha demostrado que las dietas HP mejoran la esteatosis hepática (hígado graso), sin embargo, aún son pocos los estudios que hayan analizado los efectos que la proteína de lactosuero (whey) tiene sobre el contenido graso del hígado, tanto en modelos animales como humanos[40]. Bortolotti et al.[40] analizaron el efecto que la suplementación con proteína de lactosuero tenía sobre los lípidos intra-hepáticos y los niveles plasmáticos de triglicéridos en mujeres obesas no diabéticas. Tras 4 semanas, el grupo de mujeres que consumieron la proteína de lactosuero mostraron una reducción en el contenido de lípidos intra-hepáticos (21%), y una reducción en las concentraciones de triglicéridos y colesterol plasmáticos del 15 y el 7%, respectivamente.

La reducción de la grasa corporal y plasmática observada en nuestros grupos de dieta HP ha podido ser la causa de la reducción paralela, cercana a la significación (P = 0,075), del ~25% en el porcentaje graso del hígado. Sin embargo, por el contrario, el colesterol total hepático fue mayor para los grupos de dieta HP, lo que podría sugerir la falta de relación entre niveles grasos intra-hepáticos y plasmáticos al existir una mayor concentración de ciertos ácidos grasos en el hígado[39]. Así mismo, y en concordancia a lo observado en plasma, los triglicéridos hepáticos fueron menores en los grupos HP.

A destacar es la notable interacción dieta x ejercicio observada en la reducción de las concentraciones hepáticas de triglicéridos (~60%), cuando se combina el entrenamiento de fuerza con la dieta NP. No hay mucha información acerca de los efectos del ejercicio sobre los triglicéridos hepáticos. Un estudio desarrollado en humanos concluyó que la práctica de ejercicio físico sin pérdida de peso asociada, no afectó a la composición lipídica hepática general[27]. Sin embargo, estudios desarrollados en animales aportan resultados contradictorios, algunos no han observado cambios[41] mientras otros afirman que los triglicéridos hepáticos de los animales entrenados contienen menor cantidad de ácidos grasos saturados y más insaturados (tanto mono-insaturados como poli-insaturados) que los grupos control sin entrenamiento[28,42]. Investigaciones recientes parecen estar esclareciendo dicha controversia[28,29,41,42]. El ejercicio aeróbico, pero especialmente el de fuerza (anaeróbico), parece disminuir los niveles de grasa en hígado[28], a la vez que reduce el estrés del retículo endoplasmático y la resistencia a la insulina en el tejido adiposo y hepático[29]. En nuestro grupo de ejercicio y dieta NP las concentraciones de colesterol y triglicéridos hepáticos disminuyeron en un 30 y 60%, respectivamente, frente al grupo NP sedentario. Esto nos hace presuponer que existe un mayor efecto sobre estas variables cuando se administra dieta NP y por lo tanto los niveles son, a priori, superiores. De esta forma, la combinación dieta NP y entrenamiento de fuerza se presentaría como la mejor opción de todas dentro de nuestro diseño experimental, al mejorar el perfil lipídico plasmático y hepático sin condicionar la salud hepática ni renal ni forzar al organismo a un estado de acidosis metabólica innecesario.

Algunas limitaciones deben ser mencionadas: 1) El presente estudio ha sido desarrollado en ratas y por lo tanto su extrapolación directa a humanos debe tomarse con cautela; 2) No hemos contado con instrumentación de alta tecnología para la valoración de la composición corporal, como puede ser la densitometría de absorción atómica. Por otra parte, una fortaleza del estudio ha sido el hecho de combinar grupos sedentarios con entrenamiento ante diferentes tipos de dieta, lo que nos ha permitido valorar el efecto aislado de la dieta o del ejercicio y la interacción de ambos, otorgando una buena oportunidad para investigar cómo diferentes estilos de vida pueden influir sobre importantes indicadores de salud.

Conclusiones

Los principales hallazgos del presente estudio fueron los siguientes: 1) Tanto los marcadores urinarios como plasmáticos analizados mostraron una acidosis metabólica inducida por el consumo de dietas HP, lo que podría explicar el importante incremento de peso de hígado y riñón observados en los grupos HP; 2) El entrenamiento de fuerza redujo significativamente los niveles de colesterol y triglicéridos plasmáticos, siendo este efecto mayor en el grupo de dieta HP; 3) El entrenamiento de fuerza mostró un efecto protector sobre la hipertrofia renal y hepática y la acidosis metabólica observada; 4) El porcentaje de grasa total y triglicéridos fue inferior en los grupos alimentados con dieta HP. No obstante, se observó una disminu-

ción más marcada de la concentración hepática de triglicéridos como consecuencia del ejercicio (60%) en el grupo de animales que consumió la dieta NP; 5) Se han presentado reseñables interacciones entre la dieta y el ejercicio, siendo notable el efecto protector del ejercicio sobre las variables de impacto metabólico (urea, peso del hígado y riñones) en el grupo de dieta HP.

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II. Efectos del tipo de dieta, del entrenamiento de fuerza y de la administración de esteroides anabolizantes sobre el perfil lipídico de ratas

(Artículo IV)

Effects of diet, resistance training and anabolic-androgenic steroids on body weight and lipid profile of rats

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IV

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Effects of the dietary protein amount and source, resistance training and anabolic-androgenic steroids on body weigth and lipid profile of rats

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ABSTRACT

Background: The aim of the present study was to examine the effects of the dietary protein amount and source, hypertrophy resistance training (HRT) and anabolic-androgenic steroids (AAS) on body weight and plasma and hepatic lipid profile. **Methods:** 157 adult male Wistar rats were randomly distributed in 16 experimental groups resulting in: normal-protein (NP) or high-protein (HP) diets, whey or soy-protein diets, with or without HRT and with or without AAS, for an experimental period of 3 months. **Results:** Final body weight was lower in the HRT and AAS groups compared to sedentary and non-AAS groups, respectively (all, $p<0.001$). Plasma total cholesterol (TC) was lower for the HP compared to the NP diets, for the whey compared to the soy-protein diets and for the AAS compared to the non-AAS groups (all, $p<0.001$). Plasma HDL-cholesterol was higher in the HRT groups ($p<0.05$) but lower for the AAS groups ($p<0.001$) and the HP and soy-protein diets ($p<0.05$). Plasma triglycerides (TAG) were lower for the HP diet ($p<0.001$), for the HRT ($p=0.002$) and the non-AAS groups ($p=0.001$). Liver fat was lower for the HP diet and higher for the HRT and AAS groups (all, $p<0.01$). Liver TC was lower for the NP ($p<0.01$), for the soy-protein ($p<0.05$) and for the AAS groups ($p<0.001$). Liver TAG were lower for the whey-protein diet ($p<0.001$), HRT and non-AAS groups (both, $p<0.05$). Some interactions were found, such as the greater effect of AAS on reducing body weight of rats that performed HRT or ingested a HP diet (all, $p<0.05$). HDL-C was higher when HRT was combined with HP diets ($p=0.010$) or non-AAS and when HP diets were combined with non-AAS (both, $p<0.001$). Groups that combined HRT with non-AAS administration obtained the lowest hepatic TAG ($p<0.05$). **Conclusions**: Among all the interventions tested, AAS was the factor that most negatively affected plasma and hepatic lipid profile, whereas HP diets and HRT could benefit, in general, the lipid profile, especially when combined.

Keywords: High-protein diet, anabolic-androgenic steroids, soy protein, whey protein, resistance training, plasma lipid profile, hepatic lipid profile, rats.

BACKGROUND

Obesity and abnormal lipid levels contribute significantly to the risk of coronary heart disease, a major cardiovascular disease and a serious health problem [1]. Nutritional and dietary therapy, weight loss, exercise, and scientifically proven nutritional supplementation might be appropriate to manage dyslipidemia [1-2].

High-protein (HP) diets may reduce body weight gain, fat deposition, and improve plasma lipid profile [3-6]. Furthermore, HP diets have been shown to improve hepatic lipid profile in rodent models and in humans ingesting a high-fat diet [7-9].

Several human [10-11] and rodent studies [4, 6, 12] have demonstrated the ability of whey-protein to improve body composition. Similarly, the effects of soy-protein on serum lipoproteins have been of great interest in the last decade. The new soy-based supplements may play a valuable role in reducing cardiovascular risk [13-14]. However, existing data are inconsistent or inadequate in supporting most of the suggested health benefits of consuming soy-protein [14].

Resistance training can reduce body fat, lipids and the consequent risk of cardiovascular disease [1, 15-16]. Furthermore, aerobic exercise [17-18] and, especially resistance training, could reduce fat concentration in the human liver [19] at the same time that has been shown to reduce insulin resistance in the adipose and hepatic tissue in obese rats [20].

Anabolic-androgenic steroids (AAS) were originally developed to promote growth of skeletal muscle. AAS abuse is commonly associated with bodybuilders, weightlifters, and other athletes [21]. The chronic abuse of AAS results in part in extreme alterations

in lipoproteins and apolipoproteins concentrations, especially in reducing HDL-cholesterol (HDL-C) and thus inducing an atherogenic profile with elevated risk of cardiovascular disease [22-25].

A limitation of human studies is represented by the fact that information about the intake of AAS is generally self-reported and it is hardly possible to assess the exact dosage. Furthermore, AAS are often used in combination with other complements, drugs or substances, so it is difficult to separate their toxic effects. Hence, experimental studies conducted on animal models are mandatory given the complexity of carrying out long-term and well controlled interventional studies on this topic in human subjects. Moreover, most of available evidence come from studies that examined the effect of specific interventions, e.g. focus on just exercise or just protein source in the diet. However, until date, the combined effect and interactions taking place between the dietary protein amount, protein source, resistance training and AAS-administration is unknown.

The present study aimed: 1) To examine the effects of HP vs. normal-protein (NP) diets, whey-protein vs. soy-protein diets, hypertrophy resistance training (HRT), and AAS on final body weight and plasma and hepatic lipid profile. 2) To examine potential interactions between the interventions (dietary protein amount and source, HRT, and AAS).

METHODS

Animals and experimental design

A total of 160 young albino male Wistar rats were allocated into sixteen groups derived of 4 main interventions: protein amount in the diet (HP vs. NP), protein source (whey vs. soy), training (HRT vs. sedentary), and AAS (with AAS vs. without AAS

administration) (**Figure 1**). Each specific intervention (i.e. HP diet, whey-protein diet, with HRT and with AAS) was developed in groups of 10 rats. The experimental period lasted 3 months.

The animals, with an initial body weight of 150±8 g, were housed from day 0 of the experiment in individual stainless steel metabolic cages designed for the separate collection and urine. The cages were located in a well-ventilated thermostatically controlled room (21±2ºC), with relative humidity ranging from 40 to 60%. A 12:12 reverse light-dark cycle (08.00–20.00 h) was implemented to allow exercise training during the day. Throughout the experimental period all rats had free access to double-distilled water and the animals consumed the four different diets (HP or NP, whey or soy) ad libitum. One week prior to the experimental period start, the rats were allowed to adapt to their respective diets and experimental conditions.

Body weight was measured weekly for all animals at the same time and the amount of food consumed by each rat was registered daily.

At the end of the experimental period, the animals were anaesthetized with pentobarbital and sacrificed by exsanguination by means of cannulation of the abdominal aorta. Blood was collected (with heparin as anticoagulant) and centrifuged at 3000 rpm for 15 min to separate plasma that was frozen in liquid N and stored at -80ºC.

All experiments were undertaken according to Directional Guides Related to Animal Housing and Care (European Community Council, 1986) [26], and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

Experimental diets

Formulation of the experimental diets is presented in **Table 1**. All diets were formulated to cover the nutrient requirements of rats following the recommendations of the

American Institute of Nutrition (AIN-93M) [27], with slight modifications. We have selected a 45% of protein level for the HP diet groups following previous studies in which HP diet was compared with NP diets in rats [3-4, 6, 28], whereas a 10% protein content was chosen for the NP diet groups. Commercial whey or soy-protein isolates were used as the only protein source since this protein source is widely available and used by sportsmen. Inclusion of 45% protein level in the diet was done at the expense of complex carbohydrates (wheat starch). Prior to the diet preparation, total protein concentration of the commercial whey and soy hydrolyzates and its distribution among the protein or non-protein fractions was measured. Total N content of the commercial whey-protein hydrolyzates was 11.8±0.6 g/100g of dry matter, which corresponds to a 73.8% of richness. Total N content of the commercial soy-protein hydrolyzate was 12.4±0.7 g/100g of dry matter, which corresponds to a 77.5% of richness.

Total protein concentration of the experimental diets was also assayed, with values of 44.3±2.1 % and 10.4±0.6% for the HP and NP, respectively, whey-protein diet, and 44.1±2.2% and 9.8±0.4% for the HP and NP, respectively, soy-protein diet. These values are adequate for our experimental design.

Chemical analyses

Total nitrogen (N) of the whey and soy-protein supplements and quadriceps was determined according to Kjeldahl's method. Crude protein was calculated as N x 6.25.

Plasma total cholesterol (TC), triglycerides (TAG) and HDL-C were measured using a HITACHI Roche p800 autoanalyzer.

Liver fat extraction was assessed by means of the Folch method with slight adaptations [29]. The concentration of TC and TAG in liver fat was assayed using commercial kits (Spinreact, S.A. Gerona, España).

Resistance training

The experimental groups were trained following a HRT protocol in a motorized treadmill (Panlab Treadmills for 5 rats, LE 8710R) with weights in a bag tied with a cord to the tail. This type of training was chosen in order to reproduce and mimic the type of exercise performed by people interested in gaining muscle mass and strength whose usually combine high-protein diets with AAS administration. This is important for the better interpretation of the training-derived results from this study due to the fact that perhaps we had chosen another type of exercise if our aim would have been to improve lipid profile. Therefore, our training protocol follows the established principles for human HRT, involving weights, repetitions and sets to maximize gains in muscle strength [30].

The training group exercised on alternate days. The animals ran at a constant speed of 35cm/s during the whole experimental period (12 weeks) in their dark phase. Prior to exercise training, animals were adapted to the treadmill on a daily basis for 1 week, first three days without weight and the last four days with 20% of their bodyweights. The training protocol used in the present study with slightly modifications has been previously developed and described by Aparicio et al. [31].

Animals in the control groups were managed identically to exercising animals, with the exception of exercise training. In order to avoid a possible confounding effect due to handling in the training groups, control animals were handled weekly.

Anabolic-androgenic steroids administration

Following similar studies performed in rats, the animal received 10mg/kg body weight of Nandrolone decanoate once a week by intramuscular injection in the gluteus (alternating the lateral side each week). This dose is comparable to the dose that has been reported as being frequently used by athletes (600 mg/week or approximately 8

mg/Kg/week) [32-33]. We used a commercially available nandrolone decanoate solution of 50 mg/ml (Deca-Durabolin, Organon, Oss, Netherlands).

Statistical analysis

Results are presented as mean and standard error of the mean. The effects of the dietary protein amount and source, HRT and AAS on the outcome variables were analyzed by four-ways ANOVA; with the four mentioned intervention groups as fixed factors, and values of food intakes, final body weight, quadriceps N content and plasma and hepatic lipid profile as dependent variables in separate models. Two-ways interaction terms were introduced into the models to test interactions between the following variables: HRT*dietary protein amount; AAS*dietary protein amount; AAS*HRT; AAS*protein source, and dietary protein amount*protein source. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 16.0 for Windows; SPSS Inc., Chicago, IL), and the level of significance was set at 0.05.

RESULTS

The effects of the dietary protein amount and source, HRT and AAS-administration on final body weight, food intake, quadriceps N content, and plasma and hepatic lipid profile are shown in **Table 2**.

Final body weight, food intake and quadriceps Nitrogen content

Final body weight was lower in the HRT and AAS groups compared to the sedentary and the non-AAS groups, respectively ($p<0.001$). No differences on final body weight were observed depending on the dietary protein amount or source.

Along the experimental period, food consumption gradually declined in all groups, especially from the second month (data not shown). Food intake was higher in the NP compared to the HP diet groups, for the HRT compared to the sedentary groups and for the AAS compared to the non-AAS groups (all, $p<0.01$).

We analyzed quadriceps N content since it is the musculature involved in locomotion. Quadriceps N content was higher for the HP compared to the NP diet ($p=0.001$), for the whey compared to the soy-protein diet, and for the HRT compared to the sedentary groups (both, $p<0.001$), but lower for the AAS compared to the non-AAS groups ($p<0.05$).

Plasma lipid profile

Plasma TC concentrations were lower for HP compared to NP diet groups ($p=0.001$), for the whey compared to the soy-protein and for the AAS compared to the non-AAS groups (both, $p<0.001$). Plasma HDL-C concentrations were lower in HP compared to the NP diet, for the soy compared to the whey-protein groups (both, $p<0.05$) and for the AAS compared to the non-AAS groups ($p<0.001$), but higher for the HRT compared to the sedentary groups ($p<0.05$). Plasma TAG concentrations were lower for the HP compared to the NP diet groups ($p<0.001$), for the soy compared to the whey-protein diets ($p=0.001$), for the HRT compared to the sedentary groups ($p=0.002$) and for the non-AAS compared to the AAS groups ($p=0.001$).

Hepatic lipid profile

Liver fat percentage was lower for the HP compared to the NP diet ($p=0.002$), for the sedentary compared to the HRT groups ($p=0.001$) and for the non-AAS compared to the AAS groups ($p<0.001$). Liver TC concentrations were lower for the NP compared to the HP groups ($p=0.007$), for the soy compared to the soy-protein diets ($p<0.05$) and for the AAS compared to the non-AAS groups ($p<0.001$). Liver TAG concentrations were lower for the whey compared to the soy-protein groups ($p<0.001$), for the HRT compared to the sedentary groups ($p=0.022$) and for the non-AAS compared to the AAS groups ($p=0.010$).

Interactions

Interactions found between the different interventions on final body weight are shown in **Figure 2a.** Groups that combined HRT with AAS presented a lower final body weight ($p=0.020$). The same phenomena was observed when AAS or soy-protein diets were combined with HP diets ($p=0.004$ and $p=0.032$, respectively).

Interactions observed on plasma lipid profile are shown in **Figure 2b.** Plasma HDL-C was the outcome implied in the majority of interactions. HDL-C concentrations were higher when HRT was combined with HP diets ($p=0.010$) or non-AAS administration ($p<0.001$). In the same line, HDL-C concentrations were higher when HP diets were combined with non-AAS ($p<0.001$). Groups that intake the whey-protein diets in a NP diet amount also obtained the higher levels of HDL-C ($p<0.001$). Plasma TAG concentration were higher in NP diet groups, but especially when NP diet was combined with whey-protein diets or AAS (all, $p<0.05$).

Interactions found on hepatic lipid profile are shown in **Figure 2c.** Groups that combined HRT with non-AAS administration obtained the lowest hepatic TAG concentrations ($p<0.05$). Hepatic TC was higher when whey-protein was combined with HP diets ($p<0.001$). The highest hepatic fat percentage was observed in groups fed whey NP diets ($p=0.004$).

DISCUSSION

The main findings of this study were: 1) HP diet reduced plasma TC and TAG concentrations as well as liver fat percentage. 2) Any consistent benefits on body weight loss, hepatic and plasma lipid profile have been observed derived from soy-protein instead to whey-protein consumption. 3) HRT significantly reduced body weight and increased plasma HDL-C, with a more pronounced effect in the AAS-administered and HP diets groups. HRT was also effective at reducing hepatic TAG. 4)

AAS-administration reduced final body weight, plasma and hepatic TC, but notably decreased plasma HDL-C, which could be the cause of the lower TC observed. 5) Overall the results reveal that among all the interventions tested, AAS administration was the factor that most negatively affected plasma and hepatic lipid profile, whereas HP diets and HRT could induce, in general, a better lipid profile, especially when combined.

Body weight and food intake

In contrast to what has been reported by some authors [3-4], we have not observed a lower food intake or body weight by the HP diet consumption whereas our HRT and AAS groups increased food intake and reduced body weight. Is further known that resistance training increase lean body mass and can reduce body weight [1, 15-16, 34] and therefore we have confirmed such higher muscle mass in the present study with the higher quadriceps N content observed in the HRT groups. Maybe as a direct consequence of this effect, we have observed an interaction between exercise and AAS-administration, where AAS groups with HRT also displayed a lower final body weight.

Plasma lipid profile

In agreement to our results, Noakes et al. [5] reported a greater reduction on plasma TAG concentrations in overweight women that consumed a HP diet when compared to a high-carbohydrate-low fat diet (NP diet), whereas also accordingly to us, weight loss was the same with both diets. Our HRT groups presented lower but not significant plasma TC and TAG and significant higher HDL-C concentrations, a fact that confirms the highly contrasted effects of resistance training on lipid profile [1, 15-16]. This better plasma lipid profile in general, could have a protective effect on cardiovascular diseases [1, 35].

Some studies have documented potential safety concerns on increased consumption of soy products [14, 36]. We cannot confirm lower TC concentrations after the soy-protein diet consumption under our experimental design. In fact, HDL-C was lower for the soy-protein compared to the whey-protein diet. However, TAG concentrations were lower in the soy-protein fed groups. To note is that soy-protein appears to have demonstrated effect only on reducing LDL-C [14]. Moreover, when studing the effects of soy-protein, the exact combination of active ingredients in soy products need to be identified [36]. Choquette et al. [37] aimed to analyze the combined effect of exercise and isoflavones in overweight-to-obese postmenopausal women (we do not know the specific isoflavones content in our diet). The main effects of exercise were observed for total fat mass, however, and in a similar way to what has been reported in our study, no interactions on lipid profile were observed between soy-protein and HRT.

The effects of AAS-administration on plasma lipid profile have been studied in male body builders who received a weekly intramuscular injection of nandrolone-decanoate (100 mg) or placebo for 8 weeks in a double blind way. AAS induced a ~26% decrease in HDL-C [24]. Frisch and Sumida [25], studied whether compromised serum lipoprotein concentrations would be evident in rats receiving testosterone injections over the time course of 7 weeks. No significant differences were observed between groups for any serum lipid parameters concentration. However, at week 7, serum HDL-C was significantly lower in the testosterone treated rats, compared with control animals. The authors concluded that lipoprotein profile is not altered until week 7 (our study has been performed during 12 weeks). In the study of Bonetti et al. [23] 20 male body builders, voluntarily starting AAS-administration, were followed every 6 months over 2 years. The most important long-term adverse effects were lower fertility and newly the impairment of lipid profile (especially HDL-C), associated with an increased

cardiovascular risk.

Hepatic lipid profile

Despite plasma TC was lower for HP groups, hepatic TC did not follow the same trend. A possible explanation for this lack of correlation with the lipid profile is that some fatty acids are usually present in higher amounts in the liver [38].

Recently, Bortolotti et al. [39] evaluated the effects of a whey-protein supplementation for 4 weeks on intrahepatocellular lipids and fasting plasma TAG in obese non diabetic women. Whey-protein decreased intrahepatocellular lipids by ~21%, fasting total TAG by ~15%, and TC by ~7%. The authors concluded that whey-protein reduces hepatic steatosis and improves plasma lipid profile in obese non diabetic patients, without adverse effects on glucose tolerance or creatinine clearance [39]. We have also obtained lower values of TAG among the whey-protein groups but we cannot confirm a significant hepatic TC reduction when compared to the soy-protein groups, which had slighty lower TC.

Weight loss remains the most common therapy advocated for reducing hepatic lipids in obesity and nonalcoholic fatty liver disease, whereas results regarding the effects of exercise on hepatic lipid profile are still scarce or not conclusive. Some studies have reported that hepatic TAG from trained animals contain more saturated and less unsaturated (monounsaturated as well as polyunsaturated) fatty acids than control groups without exercise [19, 40]. We have observed a very significant hepatic TAG reduction in our trained groups, especially when were combined with non-AAS administration. This concurs with the study by Johnson et al. [17], whose observed that hepatic TAG concentrations were reduced by 21% after 4 weeks of aerobic cycling exercise in obese women. The authors concluded that regular aerobic exercise reduces hepatic lipids in obesity even in the absence of body weight reduction. On the other

hand, Petridou et al. [18] examined the effects of 8 weeks of exercise training on the fatty acid composition of phospholipids and TAG in rat liver. The fatty acid composition of liver phospholipids changed with training whereas no significant differences in the fatty acid profile of hepatic TAG were found.

Hepatic TAG concentrations were higher with AAS-administration, what enphatises the adverse effect of AAS on lipid profile. A recent study has concluded that AAS could be a possible new risk factor for toxicant-associated steatohepatitis or toxicant-associated fatty liver disease development. Moreover, all cases were asymptomatic and in this type of fatty liver disease, the individuals had a low body fat mass and they did not present insulin resistance [41].

Limitation and strengths

Some limitations need to be mentioned: First, the current physiological results obtained in rodents must be confirmed in human subjects and cannot be extrapolated directly to the potential effects in humans. Second, to measure some additional lipoproteins and LDL-C would have been of interest for the interpretation of the results. On the other hand, this study involved an important number of rats, allocated in different groups so that the main effects of HP diet, HRT, the protein source and AAS-administration and the interactions taking place between them, provided a good opportunity to comprehensively investigate how these lifestyle factors and behaviors can influence dyslipidemia and the risk of coronary heart disease.

CONCLUSION

The AAS administration was the factor that most influenced plasma and hepatic lipid profile. HP diet showed a moderate positive effect on plasma lipid profile. Soy-protein did not appear to be especially effective when compared to whey-protein at promoting weight loss or improving plasma and hepatic lipid profile. The HRT performed in the

present study significantly reduced body weight and increased plasma HDL-C, with a more pronounced effect in the AAS and HP diets groups. Finally, AAS reduced final body weight, plasma and hepatic TC, but notably decreased plasma HDL-C, which could be the reason of the lower TC observed. Overall the results reveal that among all the interventions tested, AAS administration was the factor that most negatively affected plasma and hepatic lipid profile, whereas HP diets and HRT could induce, in general, a better lipid profile, especially when combined.

LIST OF ABBREVIATIONS

AAS, anabolic androgenic steroids

HDL-C, high-density lipoprotein cholesterol

HP, high protein

HRT, hypertrophy resistance training

N, nitrogen

NP, normal protein

TAG, triglycerides

TC, total cholesterol

COMPETING INTEREST

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

VAA was involved in the conception, planning and designing this study, the acquisition of data, analysis and interpretation of data, and drafting the manuscript. JMP was involved organizing this research, the acquisition of data, analysis and interpretation of data, and drafting the manuscript. EN was involved in the acquisition of data. FBO was

involved in the analysis and interpretation of data and drafting the manuscript. GK was involved in the acquisition of data. CS was involved was involved in the conception, planning and designing this study. PA was involved in the conception, planning and designing this study, the acquisition of data, organizing this research and writing the manuscript. All authors read and approved the final manuscript.

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TABLE 1. Composition of the experimental diets

Nutritional Composition (g/100g DM)	Whey protein diet		Soy protein diet	
	Normal-protein	High-protein	Normal-protein	High-protein
Whey protein supplement	13.8	63.6	-	-
Soy protein supplement	-	-	13.1	57.4
Mineral mix (AIN-93M-MX)	3.5	3.5	3.5	3.5
Vitamin mix (AIN-93-VX)	1	1	1	1
Fat (olive oil)	4	4	4	4
Choline chloride	0.25	0.25	0.25	0.25
Cellulose	5	5	5	5
Starch	61.7	22.4	62.4	28.6
Methionine	0.5	-	0.5	-
Sucrose	10	-	10	-

DM, dry matter

TABLE 2. Effects of the dietary protein amount and source, hypertrophy resistance training and anabolic-androgenic steroids (AAS) on final body weight, food intake, quadriceps N content, and plasma and hepatic lipid profile.

	Dietary protein amount			Protein source			Exercise			AAS		
	High-protein (45%)	Normal-protein (10%)	P	Whey-protein	Soy-protein	P	Hypertrophy Resistance training	Sedentary	P	AAS	Non-AAS	P
Final body weight (g)	325.1(3.9)	326.9(4.0)	0.749	328.6(4.0)	323.5(3.9)	0.362	312.0(4.3)	340.1(3.7)	<0.001	314.3(3.6)	337.8(4.3)	<0.001
Food intake (g/day)	16.6(0.2)	15.3(0.3)	0.001	16.2(0.2)	15.6(0.2)	0.125	16.5(0.2)	15.3(0.2)	0.002	16.8(0.2)	15.1(0.2)	<0.001
Quadriceps N content (g/100g DM)	14.8(0.4)	13.2(0.3)	0.001	15.1 (0.4)	13.0 (0.3)	<0.001	15.1(0.4)	12.9(0.3)	<0.001	13.4(0.3)	14.6(0.3)	0.011
Plasma lipid profile												
Plasma total cholesterol (mg/dl)	43.1(1.2)	49.4(1.1)	<0.001	42.3(1.2)	50.4(1.1)	<0.001	44.8(1.1)	47.8(1.1)	0.066	42.4(1.1)	50.3(1.2)	<0.001
Plasma HDL-cholesterol (mg/dl)	19.5(1.3)	23.8(1.3)	0.020	23.6(1.3)	19.7(1.3)	0.036	23.4(1.3)	19.8(1.3)	0.049	15.6(1.3)	27.7(1.3)	<0.001
Triglycerides (mg/dl)	44.4(3.5)	72.0(3.6)	<0.001	67.2(3.5)	49.1(3.6)	0.001	54.3(3.5)	61.6(3.7)	0.147	62.8(3.6)	53.2(3.6)	0.061
Hepatic lipid profile												
Liver fat percentage (%)	6.78(0.2)	7.80(0.2)	0.002	7.38(0.2)	7.20(0.2)	0.563	7.69(0.2)	6.74(0.2)	0.003	8.20(0.2)	6.39(0.2)	<0.001
Liver total cholesterol (mg/g)	3.96(0.1)	3.46(0.1)	0.007	3.89(0.1)	3.52(0.1)	0.048	3.62(0.1)	3.55(0.1)	0.083	3.21(0.1)	4.21(0.1)	<0.001
Liver triglycerides (mg/g)	3.10(0.2)	3.37(0.2)	0.331	2.66(0.2)	3.83(0.2)	<0.001	2.92(0.2)	3.56(0.2)	0.022	3.61(0.2)	2.87(0.2)	0.010

Values expressed as mean(standard error of the mean). DM, dry matter. HDL, high-density lipoprotein. LDL, low-density lipoprotein

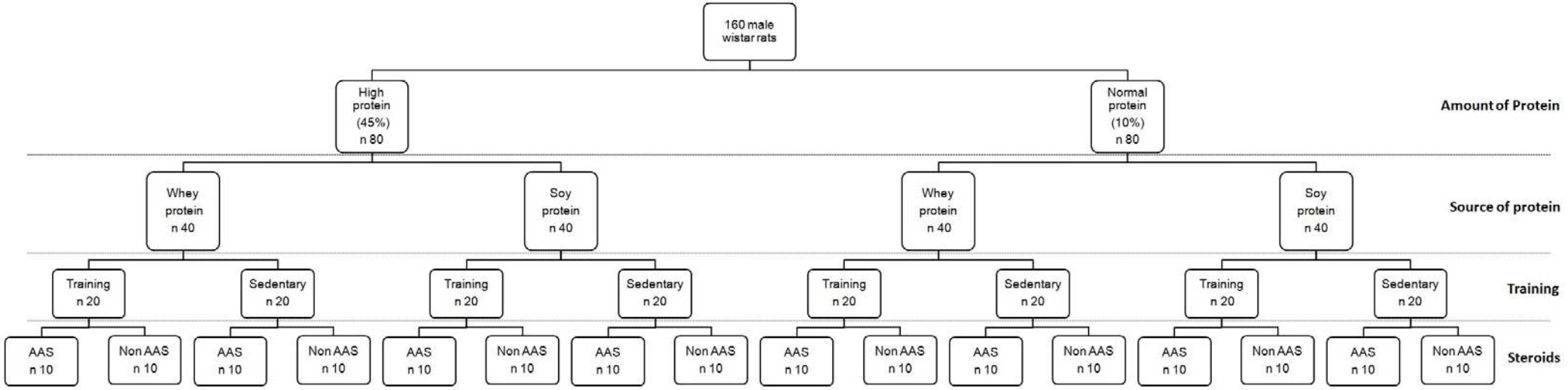


FIGURE 1. Study design showing the four different interventions: dietary protein amount (high-protein vs. normal-protein), protein source (whey vs. soy), training (resistance training vs. sedentary) and anabolic-androgenic steroids (AAS) (with AAS-administration vs. without AAS-administration).

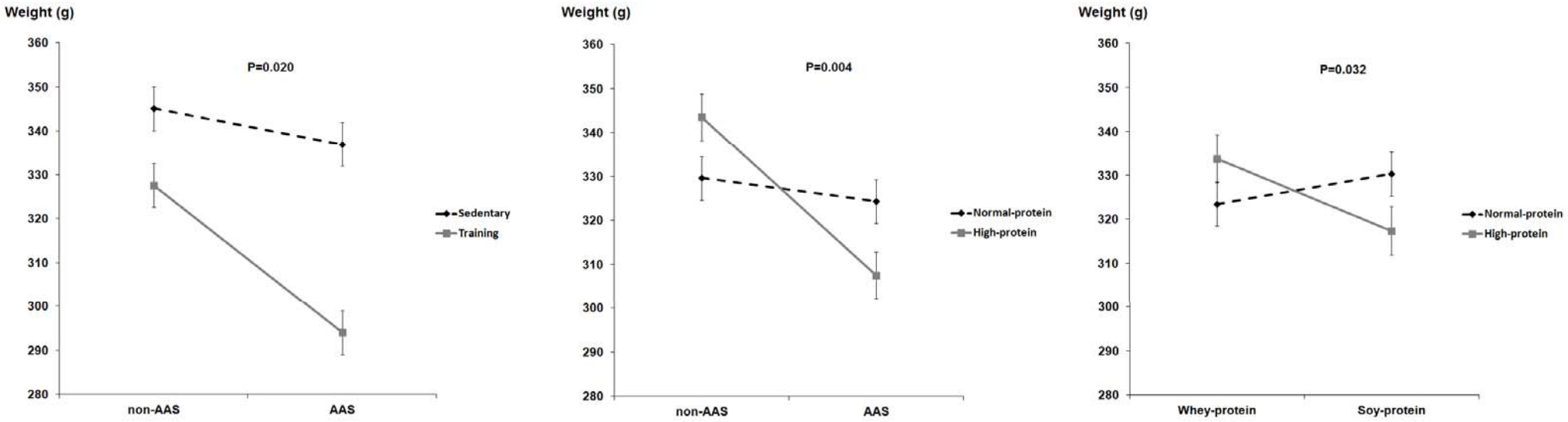
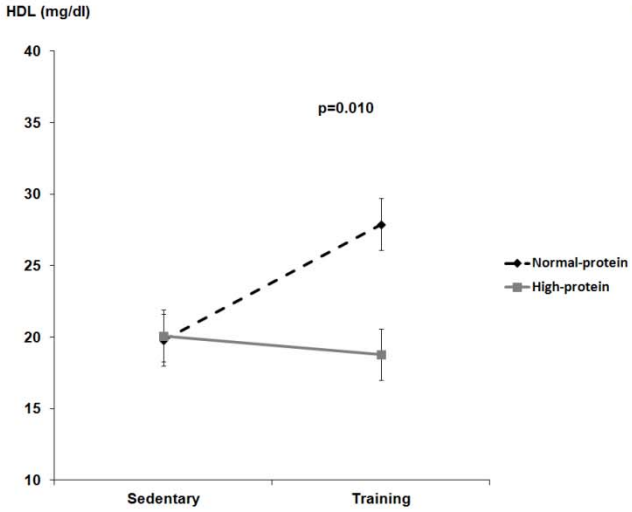
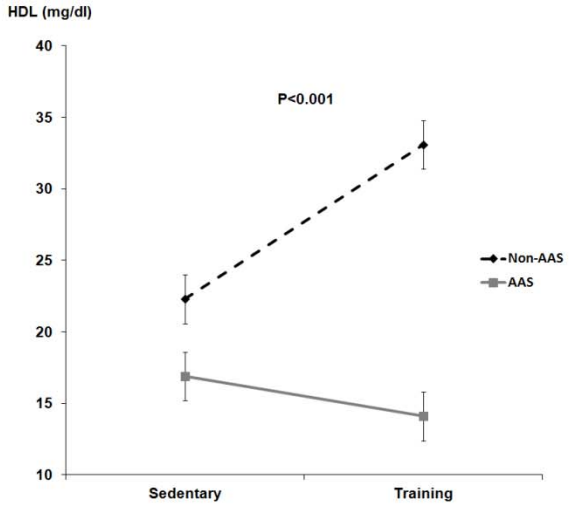
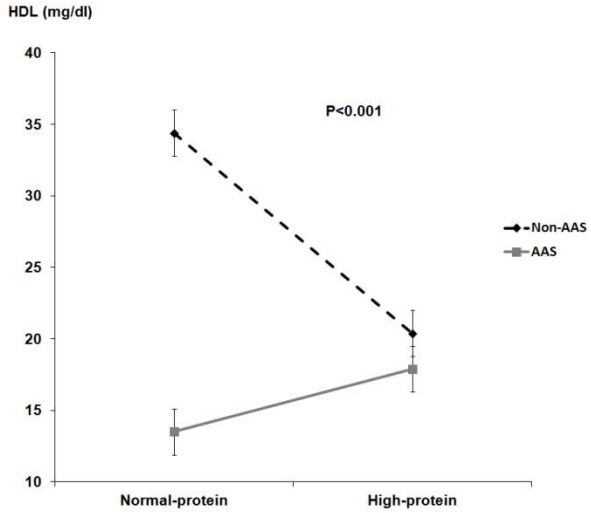
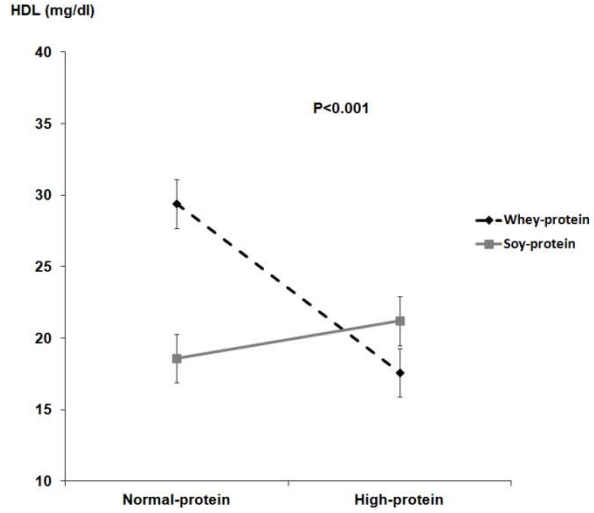


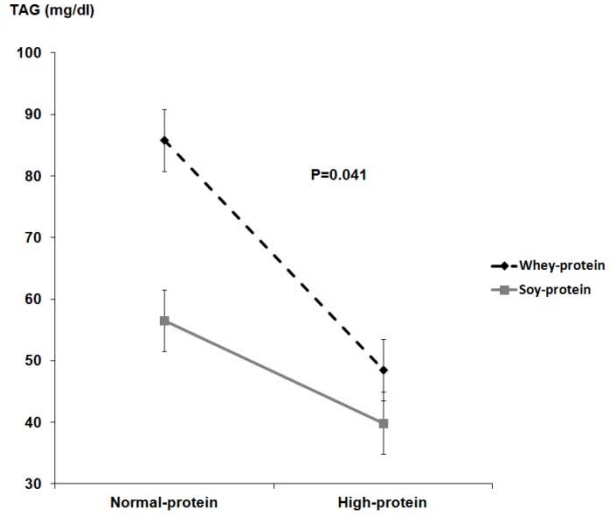
FIGURE 2A. Interactions found on final body weight. Values expressed as mean (standard error).











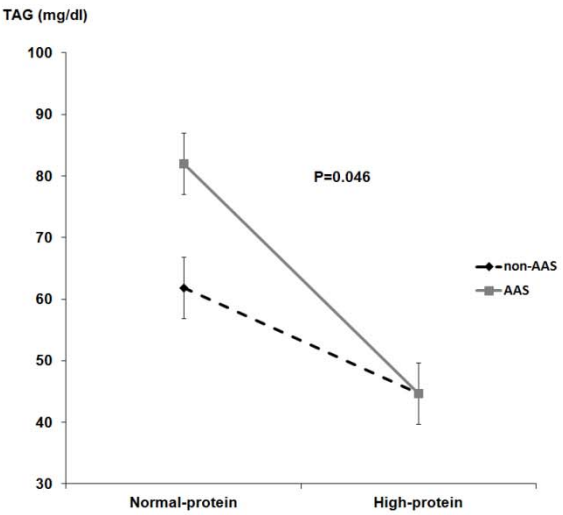


FIGURE 2B. Interactions found on plasma lipid profile. Values expressed as mean (standard error).

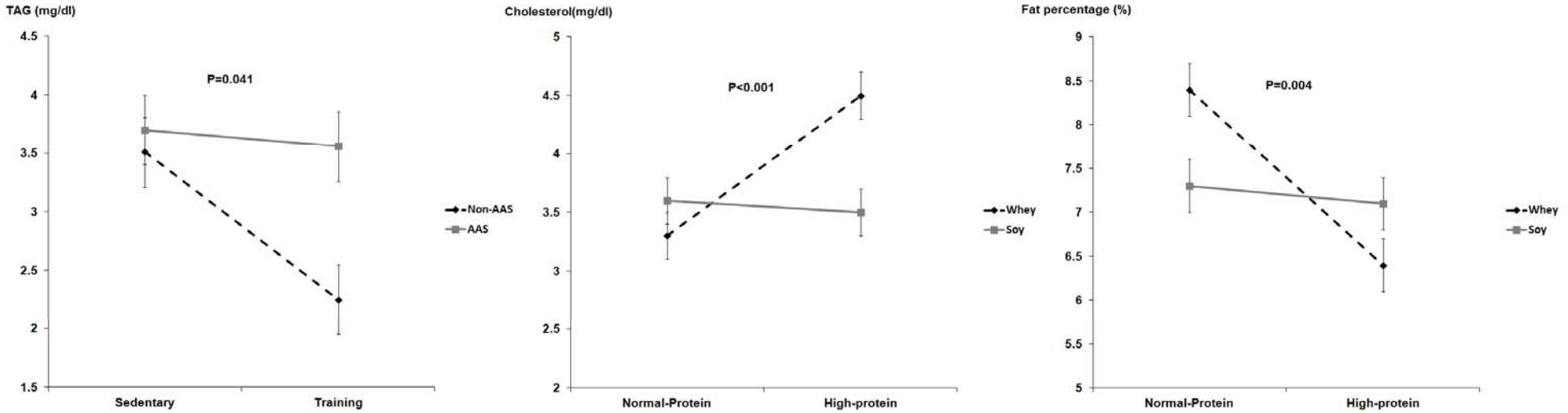


FIGURE 2C. Interactions found on hepatic lipid profile. Values expressed as mean (standard error).

III. Parámetros urinarios, plasmáticos y morfológicos del estado renal ante dietas hiperproteicas, distintas fuentes de proteína, entrenamiento de fuerza y la administración de esteroides anabolizantes

(Artículos V y VI)

Whey vs. soy protein diets and renal status in rats

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V

Whey versus soy protein diets and renal status in rats

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Abreviations:

GFR; glomerular filtration rate.

ABSTRACT

Different dietary protein sources can promote different renal status. We aimed to examine the effects of whey or soy protein intake on urinary and plasma parameters, as well as on the kidney morphology in rats. One hundred twenty Wistar rats were randomly distributed in 2 experimental groups fed whey or soy-protein diets for 12 weeks. Diets were based on commercial whey or soy protein hydrolyzates as the only source of protein. Urinary Ca was higher in the whey compared to the soy-protein group ($p<0.001$) whereas urinary citrate was lower ($p<0.001$). Urinary pH was more acidic in rats that consumed the whey compared to those consuming the soy-protein diet ($p<0.001$). No differences between groups were observed in any of the renal morphological parameters analyzed (all $p>0.05$) or other important plasma renal markers such as albumin or urea concentrations. The increase of acid and urinary Ca and the lower urinary citrate promoted by the whey-protein diet could constitute a favorable environment for nephrolithiasis and renal diseases compared to the soy-protein diet. However, no significant morphological renal changes were observed.

Key-words: soy protein, whey protein, acidosis, urine, kidney, renal morphology, rats.

1. INTRODUCTION

Whey and soy protein supplements, i.e. above 80% protein concentrates or above 90% protein isolates, have become popular among the population [1]. Whey is the liquid remaining after milk has been curdled and strained to remove the caseins. It contains proteins, lactose, vitamins, minerals, and traces of fat. Whey-protein, represents 20% of the total protein content of milk and has been reported to have utility in different applications ranging from effects on bone, muscle, blood, brain, pancreas, immune, cancer, infection, metabolism, wound healing, learning, and aging [2]. Soy protein is a vegetable high-quality protein, with a protein digestibility corrected amino acidic score of 1; it also has a high arginine/lysine ratio, which is associated with lower insulin secretion compared to animal protein. Moreover, soy protein contains isoflavones, which act as weak estrogens, inhibiting tyrosine kinase-dependent signal transduction processes and functioning as cellular antioxidants [3-4].

It has been well established that the composition of the diet affects acid-base balance. Soy protein is low in sulphur amino acids and therefore, some nutritional advantages could be obtained by replacing animal-based foods for soy foods [4]. Relative excess of animal protein ingestion (acidic load from sulphur-containing amino acids) induce intracellular acidosis [5], that stimulates hypocitraturia, which is often accompanied by hypercalciuria [5]. Both contribute to the formation of calcium-containing kidney stones [5-6].

To our knowledge, no study has analyzed in depth the effects of whey protein on renal status. The renal effects of soy protein have been widely explored, but the results are controversial and inconclusive. In fact, some studies have found a protective role of soy protein on renal health [7-9] whereas other studies failed to find significant improvements [10-11].

The present study aimed to further examine the effects of whey or soy protein intake on urinary and plasma parameters, as well as on the kidney morphology in rats.

2. METHODS AND MATERIALS

2.1. Animals and experimental design

A total of 120 young male Wistar rats were allocated into two experimental groups that consumed different protein sources (whey vs. soy) for 12 weeks. The animals, with an initial body weight of 150±8 g, were housed from day 0 of the experiment in individual stainless steel metabolism cages designed for the separate collection of faeces and urine. The cages were located in a well-ventilated thermostatically controlled room (21±2ºC), with relative humidity ranging from 40 to 60%. A 12:12 light-dark cycle (08.00–20.00 h) was implemented. Throughout the experimental period, all rats had free access to double-distilled water and the animals consumed the diets (whey or soy) ad libitum. One week prior to the experimental period, the rats were allowed to adapt to the diet and experimental conditions.

Body weight was measured weekly for all animals at the same time and the amount of food consumed by each rat was registered daily.

On week 11, a urine sample from each animal was collected for biochemical analysis. Urine volumes were recorded and samples were transferred into graduated centrifuge tubes for pH, Ca, and citrate analysis.

At the end of the experimental period, the animals were anaesthetised with pentobarbital and killed by cannulation of the abdominal aorta. Blood was collected (with heparin as anticoagulant) and centrifuged at 3000 rpm for 15 min to separate plasma that was frozen in liquid N and stored at -80ºC. Carcass weight was recorded. Kidneys were extracted, weighed and immediately the left one was introduced in formalin for the posterior histological analysis.

All experiments were undertaken according to Directional Guides Related to Animal Housing and Care (European Community Council, 1986) [12], and follow the Canadian Council on Animal Care (CCAC) guidelines. All procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

2.2. Experimental diets

Formulation of the experimental diets is presented in **Table 1**. All diets were formulated to meet the nutrient requirements of adult rats following the recommendations of the American Institute of Nutrition (AIN-93M) [13], with slight modifications. A 10% of protein content was chosen according to the American Institute of Nutrition (AIN-93M) [13]. Commercial whey or soy protein isolates were used as the only source of protein since this protein is widely available. Total N content of the commercial whey protein hydrolyzates was 11.8±0.6 g/100g of dry matter, which corresponds to a 73.8% of richness. Total N content of the commercial soy protein hydrolyzate was 12.4±0.7 g/100g of dry matter, which corresponds to a 77.5% of richness.

Total protein concentration of the experimental diets was also assayed, with values of 10.4±0.6% for the whey protein diet, and 9.8±0.4% for the soy protein diet. These values are adequate for our experimental design.

2.3. Chemical analyses

Total N of the whey and soy protein supplements was determined according to Kjeldahl's method. Crude protein was calculated as N x 6.25. Urine Ca content was determined by atomic absorption spectrophotometry using a PerkinElmer Analyst 300 spectrophotometer (PerkinElmer, Wellesley, MA, USA). Analytical results were validated by standard reference materials CRM-189, CRM-383, and CRM-709.

Urinary pH was analyzed using a bench pH-meter (Crison, Barcelona, Spain) and urinary citrate with a commercial kit (Spinreact, S.A. Gerona, España).

Plasma urea, total protein, albumin and creatinine, were measured using a Hitachi-Roche p800 autoanalyzer.

2.4. Histological analysis

Left-kidney samples were fixed in buffered 4% formalin and embedded in paraffin. Afterwards, four-micrometer-thick sections were obtained and stained with 1% Picro-sirius red F3BA (Gurr, BDH Chemicales Ltd, Poole, United Kingdom) (Sweat et al.). This technique allowed the visualization of connective fibers deep red stained on a pale yellow background [14]. The sections were assessed by optical microscopy. Forty images were captured per sample: twenty with glomerulus to determine the morphometry and the intraglomerular connective tissue and twenty from tubulointersticial area to measure the interstitial connective tissue. All images were acquired with 20x objective and analyzed with the software Fibrosis HR® [15].

2.5. Statistical analysis

Results are presented as mean and standard error of the mean. Differences between whey and soy protein groups were analyzed by ANOVA; with final body weight, food intake, urinary, plasma and renal parameters as dependent variables. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 19.0 for Windows; SPSS Inc., Chicago, IL), and the level of significance was set at 0.05.

3. RESULTS

The effects of whey and soy protein diets on final body and muscle weight, food intake and plasma and urinary parameters are shown in **Table 2**.

Food intake, final body weight, carcass and muscle weight

Along the experimental period, food consumption gradually declined in all groups,

especially from the second month (data not shown). No differences on final body weight, carcass weight, and food intake were observed between whey and soy protein groups. Gastrocnemius weight was higher in the whey protein group (p=0.029).

Plasma and urinary parameters

Plasma creatinine was lower for the whey protein diet compared to the soy protein diet (p<0.001) and plasma total protein concentration was higher (p=0.001). We have not observed differences on plasma albumin and urea concentrations between the two experimental groups tested.

Urinary Ca, as expressed in mg per litre as well as in mg per day, was higher in the whey protein compared to the soy protein diet group (both, p<0.001) whereas urinary citrate was lower (p<0.001). Urinary pH was more acidic in rats that consumed whey protein compared to those consuming soy protein diets (p<0.001).

The effects of whey and soy protein diets on kidney weight and morphology are shown in **Table 3**.

Kidney weight and morphology

Kidney wet mass, as expressed in absolute value, was lower in the whey protein diet compared to the soy protein diet (p=0.015), whereas the differences disappear when kidney wet mass is expressed related to the final body weight or carcass weight.

No differences between groups were observed in any of the renal morphological parameters analyzed (all, p>0.05).

4. DISCUSSION

Rats consuming the whey protein diet (animal source) displayed a worse urinary acid-base homeostasis profile when compared to the soy protein diet (vegetal source), which may promote a higher risk of nephrolithiasis. Despite these differences, any renal morphological changes were observed.

Renal pathologies invariably result in clinically relevant disturbances of protein metabolism. Processes regulated by the kidney are directly affected by dietary protein intake and source, especially in a state of nephropathy [16]. Consequently, limitation of ingested protein, particularly from animal sources, is crucial in order to slow the progression of chronic kidney disease and impaired renal function [16].

Of particular importance for kidney health is the maintenance of acid/base homeostasis [16]. A decrease in urinary pH, hypocitraturia and hypercalciuria, are recognized risk factors for kidney stone formation, mainly by increasing urinary saturation of calcium salts [5-6]. In our study, whey protein diet significantly increased urinary excretion of Ca, at the same time that decreased urinary pH and urinary citrate when compared to soy protein. Therefore, those animals on a whey protein diet could be at higher risk of nephrolithiasis than those consuming the soy protein diet.

Noticeable is the fact that the effects of protein also depends on the presence of other nutrients in the diet. High intakes of fruits and vegetables are associated with a reduced risk of stone formation in high-risk patients [17]. This beneficial effect of fruits and vegetables is probably due to their high content in potassium and magnesium. Potassium has been identified as a major stimulator of urinary excretion of citrate, which is an inhibitor of calcium stone formation [18]. Therefore, the alkaline content and potassium richness of fruits and vegetables are positively linked to reduced calcium excretion and reduced kidney stone formation in high-risk patients [17, 19].

Some studies have found a protective role of soy protein on renal health [7-9] whereas other studies did not [10-11]. Animal protein might promote a progressive decline in the glomerular filtration rate (GFR) of remnant kidney associated with metabolic acidosis and an endothelin-mediated increase in renal acidification. We have observed a lower urinary acidosis in the group fed the soy protein diet. In this context, Phisitkul, Hacker

et al. [9] tested whether diets that affect the acidic-base status contributes to the decline of GFR through endothelin receptors in rats with a remnant kidney. Rats on a casein diet had metabolic acidosis at baseline and developed a progressive decline in GFR after renal mass reduction. Dietary soy protein did not induce baseline metabolic acidosis and rats with remnant kidney on a soy diet had no decrease in their GFR [9]. Similarly, other studies have concluded that soy protein prevents inflammation and early nephropathic changes in rats with metabolic syndrome secondary to the attenuation of NF-kappaB activation [8]. Soy protein supplementation also improved insulin sensitivity and markedly attenuated renal basement membrane changes in fructose diet-fed rats and the authors concluded that these findings provide evidence to support the use of dietary soy protein in patients with diabetic kidney disease [7-8]. On the other hand, some authors have not observed beneficial effects when using soy protein instead of animal protein with the aim of attenuating proteinuria [10-11]. In agreement to the above mentioned authors, we have not observed differences on kidney morphology.

Limitation and strengths

The present study has several limitations that need to be mentioned. First, we have used a single source of purified protein (whey or soy), which do not reflect the human diet. Specific protein sources and amino acids have been shown to have protein-specific effects on GFR. Second, the measurement of the GFR would have been of interest in the interpretation of the present study results.

Overall, the increase of acid and urinary calcium and the decrease of urinary citrate due to the whey protein diet consumption can constitute a favorable environment for kidney stones and renal diseases, but no significant effects on kidney morphology were observed. Consequently, the assessment of the effects of different dietary protein

sources on kidney function can be useful especially for subjects at higher risk of nephrolithiasis.

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Table 1. Composition of the experimental diets

Nutritional Composition (g/100g DM)	Whey protein diet	Soy protein diet
Whey protein supplement	13.8	-
Soy protein supplement	-	13.1
Mineral mix (AIN-93M-MX)	3.5	3.5
Vitamin mix (AIN-93-VX)	1	1
Fat (olive oil)	4	4
Choline chloride	0.25	0.25
Cellulose	5	5
Starch	61.7	62.4
Methionine	0.5	0.5
Sucrose	10	10

DM, dry matter

Table 2. Effects of whey and soy protein on plasma and urinary parameters

	Source of protein		
	Whey	**Soy**	**P**
Final body weight (g)	328.5 (4.0)	324.0 (3.7)	0.408
Food intake (g/day)	16.4 (0.24)	15.8 (0.27)	0.125
Carcass weight (g)	168.2 (2.1)	173.2 (2.0)	0.090
Gastrocnemius (gN/100g DM)	14.0 (0.18)	13.2 (0.25)	0.029
Plasma Urea (mg/dl)	31.7 (1.1)	30.3 (1.1)	0.383
Plasma total proteins (g/dl)	5.60 (0.06)	5.30 (0.06)	0.001
Plasma Creatinine (mg/dl)	0.39 (0.01)	0.45 (0.01)	<0.001
Plasma Albumin (mg/dl)	2.78 (0.14)	2.69 (0.10)	0.734
Urinary Calcium (mg/L)	2.44(0.24)	0.45(0.25)	<0.001
Urinary Calcium (mg/day)	0.78 (0.07)	0.46 (0.05)	<0.001
Urinary Citrate (g/L)	0.58(0.15)	1.57(0.13)	<0.001
Urinary pH	6.34 (0.04)	6.72 (0.04)	<0.001
Urinary volume (ml)	4.03 (0.20)	3.82 (0.18)	0.443

Values expressed as mean (error standard of the mean). N, Nitrogen. DM, dry matter

Table 3. Effects of whey and soy protein on kidney morphology

	Source of protein		
	Whey	**Soy**	**P**
Kidney (g) (mean right and left)	1.00 (0.02)	1.06 (0.01)	0.015
Kidney (g/100g body weight)	0.320 (0.006)	0.333 (0.004)	0.130
Kidney (g/100g carcass)	0.602 (0.009)	0.613 (0.006)	0.377
Kidney fibrosis (%)	3.41 (0.24)	3.28 (0.16)	0.683
Kidney fibrosis area (μm²)	4456 (304)	4246 (205)	0.594
Mesangium (%)	64.9 (1.11)	63.9 (0.75)	0.457
Mesangium area (μm²)	5951 (376)	5425 (254)	0.281
Floculus I (%)	21.6 (1.36)	19.5 (0.92)	0.226
Floculus I area (μm²)	9445 (626)	8704 (423)	0.361
Floculus II (%)	45.8 (2.79)	43.3 (1.88)	0.492
Floculus II area (μm²)	19791 (1235)	19078 (834)	0.656
Glomerular area (μm²)	43779 (778)	44953 (525)	0.244
Tubular fibrosis (%)	2.47 (0.19)	2.41 (0.13)	0.807
Tubular area (μm²)	4322 (325)	4208 (219)	0.788

Values expressed as mean (error standard of the mean).

Renal effects of high-protein diets, resistance training and anabolic-androgenic steroids in rats

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Renal effects of high-protein diets, resistance training and anabolic-androgenic steroids in rats

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Running title: High-protein diets, resistance training, steroids and renal health.

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ABSTRACT

Diet, exercise and steroids can affect renal status. We examined the renal effects of high-protein (HP) diets, hypertrophy resistance training (HRT) and anabolic-androgenic steroids (AAS). Eighty Wistar rats were randomly distributed in 8 experimental groups: normal-protein (NP) or HP diets, with or without HRT and with or without AAS, for an experimental period of 3 months. Plasma creatine kinase was higher in groups that combined HP diet with HRT or AAS (p=0.001). Urinary pH was more acidic in groups that consumed HP diet and were sedentary or consumed AAS (p=0.001). Groups fed the HP diet or with AAS had heavier kidneys than those in a NP diet with or without AAS, whereas kidneys were lighter in the HRT groups (p<0.001). Plasma urea was higher for the HP compared to the NP diet (p<0.001) and for the HRT compared to the sedentary groups (p=0.001). Urinary Ca was higher for the HP compared to the NP diet, for the HRT compared to the sedentary, and for the non-AAS compared to the AAS groups (p<0.01). Glomerular area was higher in the HP compared to the NP diet (p<0.001) and for the AAS compared to the non-AAS groups (p=0.010), but HRT slightly interacted on reducing it (p=0.037). Kidney from HRT groups showed higher fibrosis, mesangium and floculus areas (p=0.005). Thus, HP diets, HRT and AAS promoted a worse renal profile and among the interventions tested, HRT induced the worst renal status. The high intensity of the training protocol performed might be on the basis of this renal affectation.

Keywords: High-protein diet, resistance training, anabolic-androgenic steroids, kidney, metabolic acidosis, renal morphology, rats.

INTRODUCTION

In the last few years, the use of high-protein (HP) diets (i.e. "The Dr. Dukan diet") is gaining in popularity among the general population. Indeed, HP diets are increasingly being recommended as one of the management strategies for weight control in overweight and obese individuals [1-2]. HP diets reduce appetite, energy intake, body weight, and fat deposition at the same time that improve plasma lipid profile [3-4]. In view of the high prevalence of obesity, type 2 diabetes, and metabolic syndrome [5], it is important to understand the effect of high levels of protein intake on health. This is particularly important for the kidney, because these patients are characterized by renal hyperfiltration and increased risk of kidney disease [6-8].

Despite the evidence of potential antiobesity effects of HP diets, the impact of such diets on renal status remains unclear [9-11]. The potentially harmful effects of dietary proteins on renal function are believed to be due to the 'overwork' induced by such diets on the kidneys. Indeed, HP diets cause elevation of glomerular filtration rate (GFR) and hyperfiltration [10]. However, some authors suggest that the link between protein-induced renal hypertrophy or hyperfiltration and the initiation of renal disease in healthy individuals has not been clearly demonstrated [12]. In fact, it has also been suggested that hyperfiltration is a normal adaptative mechanism that occurs in response to several physiological conditions [9]. Nevertheless, a few studies have observed that the exposure of rodents [13-14], cats [15] or pigs [16] to long-term HP diets results in glomerular hyperfiltration with renal morphologic injuries such as glomerular hypertrophy, and a greater prevalence of renal pathological changes.

The effects of exercise on renal status need further study. Exercise seems to reduce kidney inflammation and increase GFR and plasma albumin concentrations [17-19]. It is well established that inactivity contributes to chronic kidney disease (CKD) [20]. Exercise

improves a number of metabolic factors and reduces blood pressure and insulin resistance, which could preserve renal function [17, 20-21]. The evidence suggests that among CKD patients, the risk of remaining inactive is higher, and those patients who are weak can benefit from resistance training interventions [22]. Resistance training could increase nitrogen (N) retention and protein synthesis, ameliorates losses of muscle mass and its function, and consequently alleviates proteinuria, and thus, kidney disease in this profile of weaker population (old population, CKD, or weightlessness-exposed subjects) [22-25]. On the other hand, strenuous exercise can result in muscle damage evidenced by increased blood levels of muscle proteins such as creatine kinase (CK), lactate dehydrogenase, and myoglobin [26-27]. Renal function could be impaired when myoglobin becomes concentrated in the kidney tubules [28]. Furthermore, a significant negative correlation between plasma CK activity and the GFR indices of renal function has been observed [29].

To our knowledge, only two studies [21, 25] have analyzed kidneys morphology after an exercise intervention, and were performed in rats in weightlessness condition or with hypertension. Therefore, the morphological effects of resistance training on kidneys of animals in normal conditions remain unclear.

Anabolic androgenic steroids (AAS) are one of the most potent and widely used performance-enhancing substances among professional athletes [30-31] as well as competitive and recreational body builders or even non-athlete adolescent boys [30, 32]. Chronic use of AAS has been known to cause serious adverse effects such as liver disorders, neuropsychiatric disorders, adverse blood lipid profiles (increased LDL and decreased HDL), cardiovascular disorders, and renal complications [33-35]. Among these disorders, renal diseases have received less attention, probably because are less frequent among AAS users in comparison to other, more prevalent diseases. However, some

studies have observed cases of severe renal disorders among AAS users, especially with elevated or prolonged use [35-37].

A limitation of human studies is represented by the fact that information about the intake of AAS is generally self-reported and it is hardly possible to assess the exact dosage. Furthermore, AAS are often used in combination with other drugs or substances, so it is difficult to separate their toxic effects. Hence, experimental studies conducted on animal models are mandatory given the complexity of carrying out long-term and well-controlled interventional studies on this topic in humans.

Until date, most of available evidence come from studies that examined the effect of specific interventions, i.e. focus on just exercise or just on the amount of protein in the diet. The combined effect and interactions taking place between the dietary amount of protein, HRT and AAS-administration on renal health has never been explored. Therefore, the present study aimed to examine the renal effects of HP diets, HRT, and AAS administration in rats as well as the interactions taking place between such behaviors.

MATERIALS AND METHODS

Animals and experimental design

A total of 80 young albino male Wistar rats were allocated into eight groups derived of 3 main interventions: concentration of protein in the diet (HP vs. normal-protein (NP)), exercise (HRT vs. sedentary) and AAS (with vs. without administration). Each specific intervention (i.e. HP with exercise and with AAS) was developed in groups of 10 rats (Figure 1).

The animals, with an initial body weight of 150±8 g, were housed in individual stainless steel metabolism cages designed for the separate collection of urine. The cages were located in a well-ventilated thermostatically controlled room (21±2ºC), with relative

humidity ranging from 40 to 60%. A 12:12 reverse light-dark (08.00–20.00 h) cycle was implemented to allow exercise training during the dark period. Throughout the experimental period all rats had free access to double-distilled water and the animals consumed the two different diets (HP or normal-protein) ad libitum. One week prior to the experimental period, the rats were allowed to adapt to the diet and experimental conditions.

Body weight was measured weekly for all animals at the same time and the amount of food consumed by each rat was registered daily.

On week 11, a 12-hour urine sample from each animal was collected for biochemical analysis. Urine volumes were recorded and samples were transferred into graduated centrifuge tubes for the posterior pH, Ca, and citrate analysis.

At the end of the experimental period, the animals were anaesthetised with pentobarbital and sacrificed by cannulation of the abdominal aorta. Blood was collected (with heparin as anticoagulant) and centrifuged at 3000 rpm for 15 min to separate plasma that was frozen in liquid N and stored at -80ºC. Carcass weight was recorded. Carcass is the weight of the slaughtered animal's cold body after being skinned, bled and eviscerated, and after removal the head, the tail and the feet. Kidneys were extracted, weighed, and immediately the left one was introduced in formalin for the histological analysis.

All experiments were undertaken according to Directional Guides Related to Animal Housing and Care (European Community Council, 1986) [38], and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

Experimental diets

Formulation of the experimental diets is presented in **Table 1**. All diets were formulated to meet nutrient requirements of rats following the recommendations of the American

Institute of Nutrition (AIN-93M) [39], with slight modifications. We have selected a 45% of protein level for the HP diet groups, following previous studies in which HP diet was compared with NP diets in rats [3-4, 40], whereas a 10% of protein content was chosen for the NP diet groups. Commercial soy protein isolate was used as the only source of protein since this protein source is widely available and used by sportsmen. Inclusion of 45% protein level in the diet was done at the expense of complex carbohydrates (wheat starch). Prior to diet preparation, total protein concentration of the commercial soy hydrolyzate was measured. Total N content was 12.4±0.7 g/100g of dry matter, which corresponds to a 77.5% of richness.

Total protein concentration of the experimental diets was also assayed, with values of 44.1±2.2% and 9.8±0.4% respectively, for the HP and NP, diet. These values are adequate for our experimental design.

Resistance training

The experimental groups were trained following a HRT protocol in a motorized treadmill (Panlab TREADMILLS for 5 rats, LE 8710R) with weights in a bag tied with a cord to the tail. This type of training was chosen in order to reproduce and mimic the type of exercise performed by people interested in gaining muscle mass and strength whose usually combine high-protein diets with AAS administration. This is important for the better interpretation of the training-derived results from this study due to the fact that we would have chosen another type of exercise if our aims would have been to preserve renal function. Therefore, our training protocol follows the established principles for human HRT, involving weights, repetitions and sets to maximize gains in muscle strength [41].

The training group exercised on alternate days. The animals ran at a constant speed of 35cm/s during the whole experimental period (12 weeks) in their dark phase. Prior to

exercise training, animals were adapted to the treadmill on a daily basis for 1 week, first three days without weight and the last four days with 20% of their bodyweight. The training protocol used in the present study has been previously developed and described by Aparicio et al. [42].

Animals in the control groups were managed identically to exercising animals, with the exception of exercise training. In order to avoid a possible confounding effect due to handling in the training groups, control animals were handled weekly.

Anabolic-androgenic steroids administration

Following similar studies performed in rats, the animal received 10mg/kg body weight of Nandrolone Decanoate once a week by intramuscular injection in the gluteus (alternating the lateral side each week). This dose is comparable to the dose that has been reported as being frequently used by athletes (600 mg/week or approximately 8 mg/kg/week) [43-44]. We used a commercially available nandrolone decanoate solution of 50 mg/ml (Deca-Durabolin, Organon, Oss, Netherlands) that was diluted with peanut oil to appropriate concentrations for the lower doses to keep the volume of injection constant.

Chemical analyses

Moisture content was determined by drying to constant weight in an oven at 105±1ºC.

Total N of the soy protein supplements was determined according to Kjeldahl's method. Crude protein was calculated as N x 6.25. Insoluble N and soluble protein and non-protein N were measured using the methodology described by Periago et al. [45].

Determination of Ca in urine was performed by ICP-MS (Agilent 7500, Tokyo, Japan). All materials used in the analysis were previously cleaned with supra-pure nitric acid and ultra-pure water (18.2 Ω) obtained using a Milli Q system. Samples were prepared by digestion with nitric acid and hydrogen peroxide (supra pure quality, Merck), in a

microwave digester (Milestone, Sorisole, Italy). When the sample had been digested, the extract was collected and made up to a final volume of 10 mL for subsequent analysis. Calibration curves were prepared following the Ga addition technique as an internal standard, using stock solutions of 1000 mg/L of each element (Merck). The total Ca content in the samples was analysed using ICP-MS techniques, and the accuracy of the method was evaluated by analysis of suitable certified reference materials, Seronorm (Billingstad, Norway) and by recovery studies in samples enriched with Ca standards. The % certified value obtained for Ca was 2.5%. We used the mean of five separate determinations of this reference material.

Urinary pH was analyzed using a bench pH-meter (Crison, Barcelona, Spain). Urinary Citrate was measured using a commercial kit (Spinreact, S.A. Gerona, España).

Plasma urea, total proteins, CK and albumin were measured using a Hitachi-Roche p800 autoanalyzer.

Histological analysis

Left-kidneys samples were fixed in buffered 4% formalin and embedded in paraffin. Afterwards, four-micrometer-thick sections were obtained and stained with 1% Picro-sirius red F3BA (Gurr, BDH Chemicales Ltd, Poole, United Kingdom) (Sweat et al.). This technique allowed the visualization of connective fibers deep red stained on a pale yellow background [46].

The sections were assessed by optical microscopy. Forty images were captured per sample: twenty with glomerulus to determine the morphometry and the intraglomerular connective tissue and twenty from tubulointersticial area to measure the interstitial connective tissue. All images were acquired with 20x objective and analyzed with the software Fibrosis HR® [47].

Statistical analysis

Results are presented as mean and SEM (standard error of the mean), unless otherwise indicated, and 95%-confidence intervals for mean and for difference between means are reported. The effects of the dietary protein amount, HRT and AAS-administration on the outcome variables were analyzed by multivariate analysis of variance (MANOVA); with the three mentioned intervention groups as fixed factors, and final body weight, plasma, urinary and renal morphology parameters as dependent variables. Three-ways interaction terms were introduced into the models to test interactions between the following variables: training*diet; AAS*diet; AAS* training; AAS*training*diet.

Heavy violations of the MANOVA (and ANOVA) assumptions were not found (furthermore, possible harmful effect of variance heterogenity in the response variables is weakened by the balance on the sample size in each factor combination).

Once MANOVA signification was found, univariate ANOVA and discriminate analysis were performed in order to investigate the specific differences between groups and to evaluate which variables are important for each group separation. Finally, multiple comparisons between groups were made considering Bonferroni's method in order to control type I error propagation. When heteroscedasticity was present (by means of Levene's test) Tamahe's method was used.

All analyses were performed using the Statistical Package for Social Sciences (IBM-SPSS, version 20.0 for Windows), and the level of significance was set at 0.05.

RESULTS

The MANOVA and the interactions taking place between the three factors derived from the study design (HP diet, training and AAS) are shown in **Table 2.**

All multivariate criterions (Wilks's, Hotelling's, Pillai's and Roy's) gave the same results (only Wilk's Lambda is reported): training (Λ=0.51, $F_{14;101}$=6.8, P<0.001), diet

(Λ=0.21, $F_{14;101}$=26.4, P<0.001), and AAS (Λ=0.52, $F_{14;101}$=6.7, P<0.001) showed significant effects over the set of response variables considered. Also relevant first order interactions were found (Tables 5, 6 and 7). No higher order interactions were found.

Interactions

Due to the absence of second order (diet*HRT*AAS) significant interactions after Bonferroni´s adjustment (P<0.0036), only first order ones were explored. We have observed some HRT*AAS interactions on final body weight, with lower final body weight in groups that combined HRT with AAS compared to those sedentary without AAS (p<0.001). In the same line, the effect of HRT on final body weight reduction appears to be higher in groups that combined HRT without AAS compared to those sedentary but with AAS (p<0.001).

Some diet*AAS interactions were observed on kidneys weight (all, p<0.001). Kidneys (as expressed in mean right and left kidneys weight as well as in g/100g final body weight or g/100g carcass weight) from groups that combined HP diet with AAS were heavier than those that combined HP diet without AAS, followed by groups that combined NP diet with AAS, which also had heavier kidneys than those that combined the NP diet without AAS (Figure 2a).

Groups that combined HP diet with HRT or AAS had higher levels of plasma CK than those that combined the NP diet with HRT but without AAS (p=0.001). Similarly, groups of HP diet with HRT and AAS obtained higher levels of plasma CK than those of HP diet, sedentary and with AAS (p=0.001).

An interaction HRT*AAS was found in urinary citrate, which was lower in groups that combined HRT with steroids compared to those that combined HRT without AAS (p=0.001), (Figure 2b).

Urinary volume was higher in the sedentary groups with AAS compared to the

sedentary without AAS (p=0.002), (Figure 2b).

To the best understanding of the main effects taking place between the groups, we have divided the tables by each factor (diet, HRT and AAS). Therefore, the effects of the dietary protein amount on final body weight, plasma, urinary, and renal parameters are shown in **Table 3** and the effects of HRT and AAS on such variables are shown in **Table 4** and **Table 5**, respectively.

Main differences

Food intake, final body weight and carcass weight

Along the experimental period, food consumption gradually declined in all groups, especially from the second month (all, p<0.001, data not shown). Food intake was higher in the NP diet compared to the HP diet groups and for the non AAS-injected compared to the AAS-injected groups (both p<0.05, data not shown).

Final body weight, as well as carcass weight, were lower in the HP compared to the NP groups (p<0.001), for the HRT groups compared to the sedentary groups (p=0.002), and in the AAS groups compared to those without AAS administration (p=0.014).

Plasma and urinary parameters

Plasma urea was higher for the HP diet compared to the NP diet groups (p<0.001) and for the HRT groups (p=0.001). Plasma total proteins concentrations were higher in the HRT group compared to those sedentary (p=0.035) whereas no differences were observed attending to the rest of interventions.

Urinary Ca, as expressed by mg/L was higher for the HP compared to the NP diet (p=0.013). Urinary Ca, as expressed by mg excreted per day was higher for the HP compared to the NP diet (p=0.041), for the HRT compared to the sedentary groups (p<0.001) and for the non AAS compared to the AAS-injected groups (p<0.001).

Urinary pH was lower in rats that consumed HP diets compared to those that consumed NP diets ($p<0.001$) whereas no differences on urinary pH were observed under the other experimental conditions. Finally, urinary citrate was lower for the HP diet compared to the NP diet groups ($p<0.001$).

Renal weight and morphology

Kidney weight was involved in some diet-AAS interactions, but no training interaction was observed, therefore, the main effects of HRT over such outcome can be analyzed. Kidney wet mass weight, either expressed in absolute value or related to final body weight or carcass weight, was lower in the groups that performed HRT compared to those without HRT ($p<0.001$).

Glomerular area was higher in the HP diet compared to the NP diet groups ($p<0.001$) and for the AAS-administrated groups compared to those without AAS ($p=0.010$) but without differences derived from the HRT.

Kidneys from HRT groups showed higher kidney fibrosis, mesangium and floculus areas and percentages, and a higher tubular fibrosis ($p=0.005$).

DISCUSSION

HP diets, HRT and AAS-administration promoted, in general, a worse urinary, plasma and renal profile. Among all the intervention, the intake of HP diets was the factor that most affected the plasma and urinary parameters of metabolic acidosis studied in the present study. Therefore, HP diets may negatively affect the acid-base balance and could increase the risk of nephrolithiasis or other renal diseases. HRT promoted a lower renal inflammation, as measured by kidney weight, but induced renal and tubular fibrosis. AAS-administration and HP diets slightly increased glomerular area but without modifying renal and tubular fibrosis. Among the interventions tested, the HRT

protocol performed promoted, in general, the worst renal profile. The high intensity of the type of training performed in the present study (hypertrophy strength) can be the cause of this renal affectation.

As expected, and in agreement to what has been reported by other authors, HP diets reduced energy intake and body weight, which could improve plasma lipid profile [3-4]. However, excessive protein consumption might promote renal damage by chronically increasing net filtration pressure and hyperfiltration [11]. We have confirmed, under our experimental conditions, that plasma urea concentration increases when HP diets are consumed [10, 42, 48-49]. More urea needs to be filtered, because more of it has to be excreted, and this could be the cause of the ~10% higher glomerular area observed in our groups fed the HP diet. Frank et al. [10], detected that blood urea nitrogen, serum uric acid, glucagon, natriuresis, urinary albumin, and urea excretion increased significantly when young men consumed a HP diet, and concluded that more attention should be paid to the potential adverse renal effects of HP diets.

Accordingly to the evidence reported previously by our group [42] and by other studies [16, 50-52] we have also observed an increase on kidney weight after 12 weeks of HP diet consumption. This renal inflammation could be induced via release of proinflammatory chemokines, such as renal monocyte chemoattractant protein-1 (MCP-1), which plays an important role in the recruitment of inflammatory cells into the kidney [53]. Infiltrating inflammatory cells interact with renal cells, causing them to synthesize excessive extracellular matrix, ultimately resulting in the development of kidney fibrosis [53-56]. Some studies performed in rodents [50-51] or pigs [16] have also observed histological damage with HP diets in the long term. In the study by Jia et al. [16], female pigs received either NP or HP (15 or 35%) isocaloric diets. The HP compared with NP diet resulted in enlarged kidneys. Renal and glomerular volumes were 60–70% higher by the end of the

study. These enlarged kidneys had greater evidence of histological damage, with 55% more fibrosis and 30% more glomerulosclerosis. Furthermore, plasma concentrations of homocysteine and MCP-1 were extremely higher in the HP-fed groups [16]. Interestingly, we have observed a significantly higher (9%) glomerular area in the HP diet groups but we did not detect renal or tubular fibrosis in rats fed the HP diet. This concurs to what has been reported by others authors who observed that in long interventional studies, including overweight or obese healthy subjects, without preexisting renal dysfunction, the HP diet did not adversely affect renal function, either it increased GFR and kidney size [57] or it did not [58].

Of particular importance for kidneys health is the maintenance of urinary acid/base homeostasis [59]. Relative excess of animal protein ingestion (acid load from sulphur-containing amino acids) might produce intracellular acidosis [60]. Intracellular acidosis stimulates urinary hypocitraturia, that is often accompanied by urinary hypercalciuria [60]. A decrease on urinary pH, hypocitraturia and hypercalciuria, are recognized risk factors for kidney stone formation [40, 60]. In our study, the HP diet significantly increased urinary excretion of Ca, at the same time that decreased urinary pH and citrate, therefore, those animals could be at a higher risk of nephrolithiasis.

Taken together, these results suggest that HP diets might not have an adverse effect on healthy people, but may accelerate renal diseases in people with renal dysfunction [12] or in higher risk of nephrolithiasis [40, 60].

Patients with chronic renal failure usually present the syndrome of "protein-energy malnutrition", which requires early detection and vigorous treatment [59]. For these patients, the risk of remaining inactive is higher, and those who are weak can benefit from resistance training interventions [22]. A recent study explored the effects of long-term weightlessness on the renal tissue and investigated the simulated microgravity on

the renal morphological damages and related molecular mechanisms in rats. HRT reduced kidney cell apoptosis and expression of HSP70 protein and attenuated the kidney impairment imposed by weightlessness [21, 25]. In a study performed in spontaneously hypertensive rats, exercise training delayed hypertension, prevented oxidative stress and inflammation, preserved antioxidant status, prevented an increase in circulating AngII levels, and preserved renal hemodynamics and structure. In addition, exercise-induced effects, at least, in part, were found to be pressure-independent [21] .

In the general healthy population, resistance training decreases C-reactive protein, increases insulin sensitivity, decreases body fat content, increases insulin-like growth factor-1 (IGF-1), and decreases microalbuminuria [17-19]. Moreover, in the nondialysis CKD population, resistance training has been reported to reduce renal inflammation, increase plasma albumin concentrations and GFR, maintain body weight, increase muscle strength and IGF-1 [17-19]. In addition to the lower body weight, we can confirm a lower renal inflammation promoted by the HRT by means of the lower kidney weight observed in our trained animals.

Surprisingly, despite this reduction on kidney weight found in our resistance trained groups that consumed HP diet or were injected with AAS, the rest of morphological renal variables studied showed, in general, a worse profile, with higher renal fibrosis, mesangiums and floculus as well as tubular fibrosis. Different hypotheses can explain these findings: 1) Rhabdomyolysis causes renal dysfunction associated with renal vasoconstriction, tubular toxicity and luminal obstruction [61]. Renal filtration of metmyoglobin released from damaged muscle, and that is filtered at the glomerulus, is known to cause acute renal injury in exercise rhabdomyolysis [62-64]. Levels of methaemoglobin increase during high intensity exercise, while levels of antioxidants, such as glutathione, decrease [62]. Moreover, there is now accumulating evidence that

renal injury, caused by lipid peroxidation, is important in the pathogenesis of renal failure. Current data have shown that the heme center of myoglobin can initiate lipid peroxidation and renal injury without invoking release of free iron, and this process is due to redox cycling of the heme group from ferrous to ferric and to ferryl oxidation states. Alkaline conditions prevent myoglobin-induced lipid peroxidation by stabilizing the reactive ferryl myoglobin complex [28]. During heavy physical exercise (as the performed in our HRT groups), two phenomena are concomitant: the decrease of GFR and the release into the blood of some molecules from muscles. The acute decrease of GFR is linked to the reduction of renal blood flow and has been described in marathon runners and cyclists [65]. The increase of myoglobin in the blood is linked to muscular damage from increased permeability of or damage to cellular membranes. The muscular-derived molecules are usually cleared from blood by the reticulo-endothelial system, except for myoglobin that is cleared by the kidneys. Myoglobin is usually filtered and excreted in urine, but renal function could be impaired when myoglobin becomes concentrated in the kidney tubules. We have detected higher levels of CK in our resistance-trained groups when HRT was accompanied by the AAS administration. A ten-fold increase of CK is common in athletes after exercise, even professional athletes [66]. On the other hand, a recent study has found significant inverse correlations between serum CK activity and the GFR indexes of renal function [29]. 2) Cortisol is a glucocorticoid released from the adrenal cortex in response to stress which is believed to play a role in the remodeling of tissue [67] in response to intense exercise [68]. In fact, resistance exercise protocols that stimulate the greatest lactate response (as ours) seem to cause the greatest elevations in cortisol [69-70]. Furthermore, protocols that result in the greatest concentrations of circulating CK 24-hours post-exercise, also result in the greatest elevations in circulating cortisol [71]. In a recent study [72], rats ran for 60 min/d, 5

d/wk at 25 m/min and 0% grade. Plasma corticosterone levels increased in trained group when compared with control group ($p<0.003$). Sustained delivery of supraphysiological levels of corticosterone play a role on modifying kidney structure and function [73]. 3) We have also observed a significant higher total plasma proteins concentration in our trained groups, which could promote hyperproteinuria. Proteins filtered by the glomerulus cause injury of tubular cells, leading to progressive tubulointerstitial fibrosis [74]. When proximal tubular cells are exposed to excessive amounts of protein, a variety of harmful responses are initiated [74-77]. 4) Finally, disturbances promoted by intense resistance training, such as hypoxia, glucose depletion or oxidative stress may lead to endoplasmic reticulum (ER) dysfunction, which can induce ER stress. Accumulating evidence indicates that ER stress contributes to glomerular and tubular damage in patients with acute and CKD [78-79].

Chronic use of AAS has been known to cause serious adverse health effects [33-35]. Among these disorders, renal diseases have received less attention, probably because are less frequent among AAS users, where altered lipid profile and hepatotoxicity have been further explored [35]. However, study-cases have showed renal damage after anabolic steroid abuse, especially with elevated or prolonged use of AAS [35-37]. Acute kidney injuries include focal segmental glomerulosclerosis, glomerulomegaly, tubular atrophy and interstitial fibrosis [36], inflammatory interstitial nephritis, and acute tubular necrosis [37]. Much of the knowledge of these potentially severe but usually limited side effects is confounded by use of combinations of different steroid preparations and by the concomitant use with other substances [35]. Under our experimental conditions, we have appreciated a higher kidney weight and glomerular area in the AAS-administrated groups, especially when AAS were combined with HP diets.

Limitation and strengths

The present study has several limitations that need to be mentioned. First, specific protein sources and amino acids have been shown to have unique effects on GFR[11] and we have used only soy protein. Second, to measure some proinflammatory chemokines, such as MCP-1, which plays an important role in the recruitment of inflammatory cells into the kidney, as well as myoglobin and corticosterone would have been of interest in the interpretation of this study results, especially among the HRT groups.

A positive note is the fact that this study involved an important number of rats, allocated in different groups, so that the effects of HP diet, HRT, and AAS administration under the same experimental model provides a good opportunity to comprehensively investigate how these lifestyle factors can influence some important renal health-related outcomes.

CONCLUSIONS

In conclusion, HP diets, HRT and AAS-administration promoted, in general, a worse urinary, plasma and renal profile. HP diets might negatively affect acid-base balance, constituting a favorable environment for nephrolithiasis and renal diseases in high-risk people, but no clear harmful effect of HP diets on renal morphology has been found and HP diets might be deleterious only in patients with a preexisting metabolic renal dysfunction. Under our experimental design, among the interventions tested, HRT promoted the worst renal profile. The high intensity of the type of resistance training performed in the present study (hypertrophy strength) can be the cause of this renal deterioration.

Some conclusions regarding exercise intensity and type could be extracted. The volume and intensity of the exercise performed for a better renal health should be adapted to each person/patient profile. Those persons with a noticeable muscle weakness could benefit from resistance training interventions, whereas patients with hypertension or

without sarcopenia could obtain more benefits just with low-moderate intensity aerobic exercise. Future studies will confirm or contrast the present study results.

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Table 1. Composition of the experimental diets

Nutritional Composition (g/100g DM)	**Normal-protein**	**High-protein**
Soy protein supplement	13.1	57.4
Mineral mix (AIN-93M-MX)	3.5	3.5
Vitamin mix (AIN-93-VX)	1	1
Fat (olive oil)	4	4
Choline chloride	0.25	0.25
Cellulose	5	5
Starch	62.4	28.6
Methionine	0.5	-
Sucrose	10	-

DM, dry matter

Table 2. Multivariate analysis of variance (MANOVA) and the interactions taking place between the three factors derived from the study design (diet, training and anabolic-androgenic steroids (AAS))

	Diet * Training		**Diet * AAS**		**Training * AAS**		**Diet * Training * AAS**	
Wilks Lambda	L=0.719		L=0.696		L=0.611		L=0.767	
	F	P	F	P	F	P	F	P
Urinary Citrate (g/L)	3.048	0.084	3.513	0.063	10.853	**0.001**	0.027	0.869
Urinary Calcium (mg/L)	0.717	0.399	0.387	0.535	1.953	0.165	1.097	0.297
Urinary Calcium (mg/day)	0.297	0.587	2.671	0.105	1.638	0.203	3.975	0.049
Plasma Urea (mg/dl)	0.035	0.852	6.698	0.011	0.097	0.756	0.565	0.454
Plasma total proteins (g/dl)	0.105	0.747	0.130	0.719	2.340	0.129	2.839	0.095
Creatin Kinase (uL)	17.460	**0.000**	7.251	0.008	12.516	**0.001**	0.668	0.415
Urinary volumen (ml)	0.137	0.712	0.328	0.568	10.137	**0.002**	0.046	0.830
Urinary pH	5.074	0.026	2.665	0.105	1.506	0.222	1.023	0.314
Glomerular area (µm²)	0.008	0.929	0.811	0.370	1.205	0.275	4.439	0.037
Factor1	0.899	0.345	0.365	0.547	0.035	0.851	1.800	0.182
Factor2(1)	2.553	0.113	13.577	**0.000**	0.526	0.470	0.515	0.474
Factor2(2)	0.524	0.470	0.541	0.464	4.969	0.028	5.673	0.019

The estimated Bonferroni´s signification (P<0.0036) is marked in blond numbers.
Factor 1 corresponds to kidney fibrosis (%), kidney fibrosis area (µm²), mesangium (%), mesangium area (µm²), floculus I (%), floculus I area (µm²), floculus II (%), floculus II area (µm²), tubular fibrosis (%) and tubular area (µm²).
Factor 2(1) corresponds to kidney (g) (mean right and left), kidney (g/100g body weight) and kidney (g/100 g carcass).
Factor 2(2) corresponds to final body weight and carcass weight.

Table 3. Main effects of the dietary protein amount on body weight, plasma, urinary and renal parameters

Diet L=0.215			**Normal Protein**		**High Protein**	
F(14;101)=26.4 (P<0.001)	**F**	**P**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**
Final body weight (g)	37.725	0.000	328.5 (3.9)	(320.6; 336.3)	314.3 (4.0)	(306.3; 322.2)
Carcass weight (g)			173.9 (2.1)	(169.7; 178.0)	168.1 (2.1)	(163.8; 172.3)
Plasma Urea (mg/dl)	21.150	<0.001	26.1 (1.1)	(23.9; 28.2)	34.6 (1.1)	(32.4; 36.8)
Plasma total proteins (g/dl)	3.507	0.064	5.42 (0.068)	(5.29; 5.56)	5.22 (0.07)	(5.08; 5.36)
Plasma Creatine kinase (u/L)	0.592	0.443	1160 (121)	(921; 1401)	1146 (123)	(902; 1391)
Urinary Citrate (g/L)	64.290	<0.001	2.42 (0.16)	(2.11; 2.74)	0.79 (0.16)	(0.47; 1.11)
Urinary Calcium (mg/L)	6.424	0.013	2.10 (0.09)	(1.91; 2.28)	2.46 (0.09)	(2.27; 2.64)
Urinary Calcium (mg/day)	8.351	0.005	0.50 (0.05)	(0.39; 0.60)	0.76 (0.05)	(0.65; 0.87)
Urinary volume (ml)	0.592	0.443	3.71 (0.23)	(3.25; 4.16)	4.45 (0.24)	(3.98; 4.91)
Urinary pH	53.703	0.000	6.91 (0.05)	(6.80; 7.02)	6.37 (0.05)	(6.26; 6.48)
Kidney (g) (mean R and L)	65.051	<0.001	0.97 (0.02)	(0.94; 1.01)	1.10 (0.02)	(1.07; 1.13)
Kidney (g/100g body weight)			0.30 (0.004)	(0.29; 0.31)	0.35 (0.004)	(0.34; 0.36)
Kidney (g/100g carcass)			0.56 (0.006)	(0.55; 0.57)	0.65 (0.007)	(0.64; 0.67)
Kidney fibrosis (%)	0.579	0.448	3.33 (0.17)	(3.00; 3.67)	3.37 (0.17)	(3.04; 3.71)
Kidney fibrosis area (µm²)			4406 (217)	(3976; 4835)	4336 (220)	(3898; 4773)
Mesangium (%)			64.5 (0.77)	(62.8; 65.9)	63.5 (0.79)	(62.0; 65.1)
Mesangium area (µm²)			5257 (264)	(4734; 5780)	5898 (268)	(5366; 6431)
Floculus I (%)			20.0 (0.97)	(18.1; 21.9)	20.6 (0.9)	(18.7; 22.6)
Floculus I area (µm²)			8410 (440)	(7537; 9282)	9439 (448)	(8550; 10326)
Floculus II (%)			44.8 (1.9)	(40.8; 48.7)	44.0 (2.0)	(40.1; 48.0)
Floculus II area (µm²)			18581 (868)	(16862; 20300)	19953 (883)	(18204; 21702)
Tubular fibrosis (%)			2.53 (0.13)	(2.27; 2.79)	2.32 (0.13)	(2.06; 2.59)
Glomerular area (µm²)	25.515	0.000	42338 (536)	(41275; 43399)	46013 (545)	(44932; 47093)

Values expressed as mean (standard error of the mean); CI, confident interval; R, right; L, left

Table 4. Main effects of resistance training on body weight, plasma, urinary and renal parameters

Training L=0.514			**Sedentary**		**Training**	
F(14;101)=6.8 (P<0.001)	**F**	**P**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**
Final body weight (g)	9.817	0.002	338.0 (3.9)	(330.3; 345.8)	304.7 (4.0)	(296.7; 312.7)
Carcass weight (g)			178.0 (2.1)	(173.9; 182.1)	163.9 (2.2)	(159.7; 168.2)
Plasma Urea (mg/dl)	11.650	0.001	27.6 (1.1)	(25.5; 29.7)	33.1 (1.1)	(30.9; 35.3)
Plasma total proteins (g/dl)	4.553	0.035	5.19 (0.07)	(5.05; 5.32)	5.45 (0.07)	(5.31; 5.59)
Plasma Creatine kinase (u/L)	0.605	0.438	1012 (120)	(774; 1251)	1294 (124)	(1049; 1540)
Urinary Citrate (g/L)	4.167	0.044	1.95 (0.16)	(1.64; 2.27)	1.25 (0.16)	(0.93; 1.57)
Urinary Calcium (mg/L)	1.978	0.162	2.16 (0.09)	(1.98; 2.34)	2.40 (0.09)	(2.22; 2.59)
Urinary Calcium (mg/day)	4.263	0.041	0.56 (0.05)	(0.46; 0.67)	0.69 (0.05)	(0.58; 0.80)
Urinary volume (ml)	0.638	0.426	4.12 (0.23)	(3.66; 4.57)	4.04 (0.24)	(3.57; 4.51)
Urinary pH	0.613	0.435	6.66 (0.05)	(6.55; 6.76)	6.62 (0.05)	(6.51; 6.73)
Kidney (g) (mean R and L)	19.470	<0.001	1.09 (0.01)	(1.06; 1.12)	0.98 (0.01)	(0.95; 1.01)
Kidney (g/100g body weight)			0.33 (0.004)	(0.32; 0.33)	0.32 (0.005)	(0.32; 0.33)
Kidney (g/100g carcass)			0.61 (0.006)	(0.60; 0.63)	0.60 (0.007)	(0.59; 0.61)
Kidney fibrosis (%)	8.060	0.005	2.99 (0.16)	(2.67; 3.32)	3.71 (0.17)	(3.38; 4.05)
Kidney fibrosis area (µm²)			3869 (216)	(3442; 4297)	4872 (222)	(4432; 5312)
Mesangium (%)			64.7 (0.8)	(63.2; 66.2)	63.2 (0.8)	(61.6; 64.8)
Mesangium area (µm²)			5201 (262)	(4681; 5721)	5954 (270)	(5419; 6489)
Floculus I (%)			18.5 (0.9)	(16.6; 20.5)	22.1 (0.9)	(20.2; 24.1)
Floculus I area (µm²)			8338 (438)	(7470; 9206)	9510 (451)	(8617; 10403)
Floculus II (%)			39.7 (1.9)	(35.8; 43.6)	49.1 (2.0)	(45.1; 53.1)
Floculus II area (µm²)			17716 (863)	(16007; 19426)	20817 (888)	(19058; 22577)
Tubular fibrosis (%)			2.08 (0.13)	(1.82; 2.34)	2.78 (0.13)	(2.51; 3.04)
Glomerular area (µm²)	3.707	0.057	45056 (533)	(44000; 46112)	43293 (548)	(42206; 44380)

Values expressed as mean (standard error of the mean); CI, confident interval; R, right; L, left.

Table 5. Main effects of the anabolic androgenic steroids (AAS) administration on body weight, plasma, urinary and renal parameters

Steroids L=0.52			**Non-AAS**		**AAS**	
F(14;101)=6.7 (P<0.001)	**F**	**P**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**
Final body weight (g)	6.190	0.014	327.0 (4.7)	(317.7; 336.4)	315.7 (3.4)	(309.1; 322.3)
Carcass weight (g)			170.3 (2.5)	(165.3; 175.3)	171.6 (1.7)	(168.1; 175.2)
Plasma Urea (mg/dl)	1.060	0.305	29.6 (1.3)	(27.0; 32.2)	31.1 (0.9)	(29.3; 32.9)
Plasma total proteins (g/dl)	0.684	0.410	5.28 (0.08)	(5.12; 5.44)	5.36 (0.06)	(5.24; 5.47)
Plasma Creatine kinase (u/L)	11.782	0.001	905 (144)	(619; 1192)	1401 (103)	(1196; 1605)
Urinary Citrate (g/L)	1.920	0.169	1.75 (0.19)	(1.38; 2.13)	1.45 (0.14)	(1.19; 1.72)
Urinary Calcium (mg/L)	3.788	0.054	2.41 (0.11)	(2.20; 2.63)	2.14 (0.08)	(1.99; 2.30)
Urinary Calcium (mg/day)	15.603	<0.001	0.78 (0.06)	(0.65; 0.91)	0.48 (0.05)	(0.39; 0.57)
Urinary volume (ml)	0.291	0.591	3.97 (0.28)	(3.42; 4.52)	4.19 (0.20)	(3.80; 4.58)
Urinary pH	1.875	0.174	6.70 (0.06)	(6.57; 6.82)	6.58 (0.05)	(6.49; 6.67)
Kidney (g) (mean R and L)	6.189	0.014	1.01 (0.02)	(0.98; 1.05)	1.06 (0.01)	(1.04; 1.09)
Kidney (g/100g body weight)			0.31 (0.005)	(0.30; 0.32)	0.34 (0.004)	(0.33; 0.35)
Kidney (g/100g carcass)			0.59 (0.008)	(0.58; 0.61)	0.62 (0.005)	(0.61; 0.63)
Kidney fibrosis (%)	0.128	0.721	3.41 (0.2)	(3.01; 3.80)	3.30 (0.14)	(3.02; 3.58)
Kidney fibrosis area (μm²)			4477 (259)	(3963; 4990)	4265 (184)	(3900; 4631)
Mesangium (%)			62.9 (0.92)	(61.1; 64.8)	65.0 (0.66)	(63.7; 66.3)
Mesangium area (μm²)			5516 (315)	(4891; 6141)	5640 (224)	(5194; 6084)
Floculus I (%)			20.6 (1.2)	(18.3; 22.9)	20.1 (0.8)	(18.4; 21.7)
Floculus I area (μm²)			8866 (526)	(7823; 9908)	8983 (374)	(8240; 9725)
Floculus II (%)			45.4 (2.4)	(40.8; 50.1)	43.4 (1.7)	(40.1; 46.7)
Floculus II area (μm²)			19340 (1037)	(17286; 21394)	19194 (738)	(17732; 20656)
Tubular fibrosis (%)			2.40 (0.16)	(2.09; 2.71)	2.46 (0.11)	(2.24; 2.68)
Glomerular area (μm²)	6.905	0.010	43131 (640)	(41863; 44401)	45218 (456)	(44315; 46122)

Values expressed as mean (standard error of the mean); CI, confident interval; R, right; L, left.

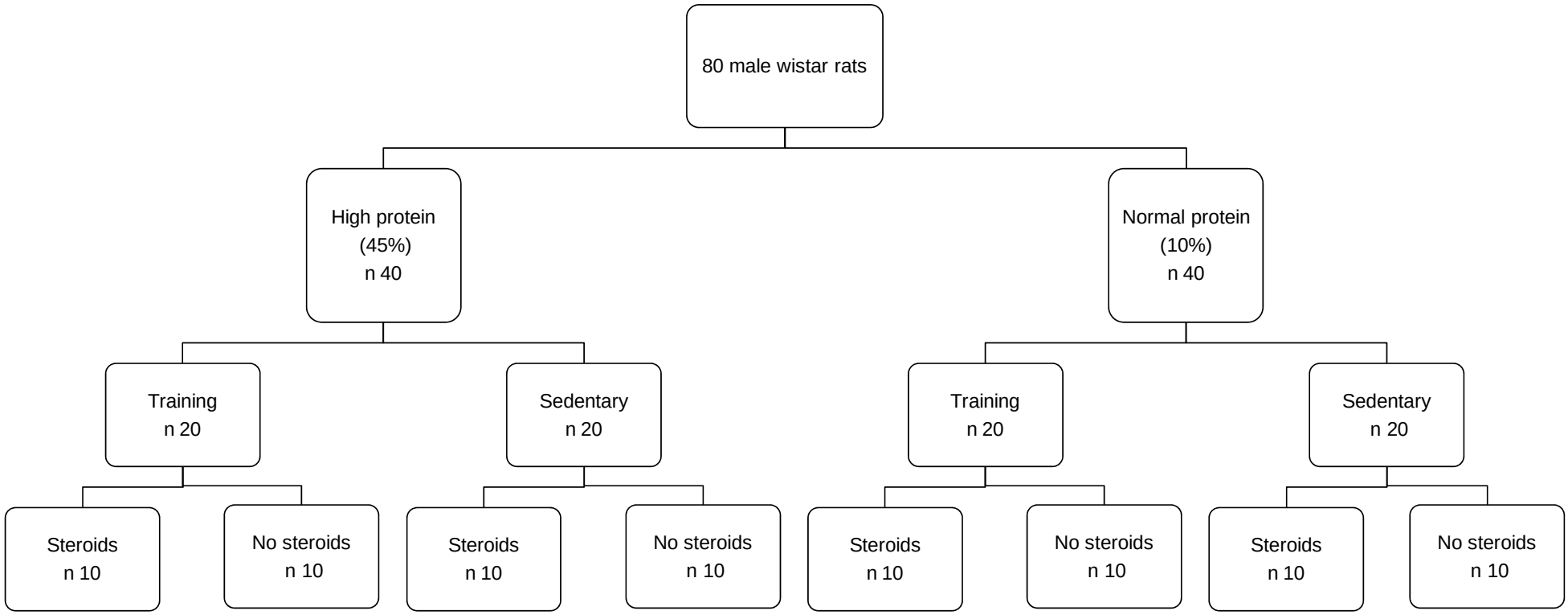

Figure 1. Study design showing the three different interventions: amount of protein in the diet (high-protein vs. normal-protein), training (resistance training vs. sedentary) and anabolic-androgenic steroids (with vs. without steroids)

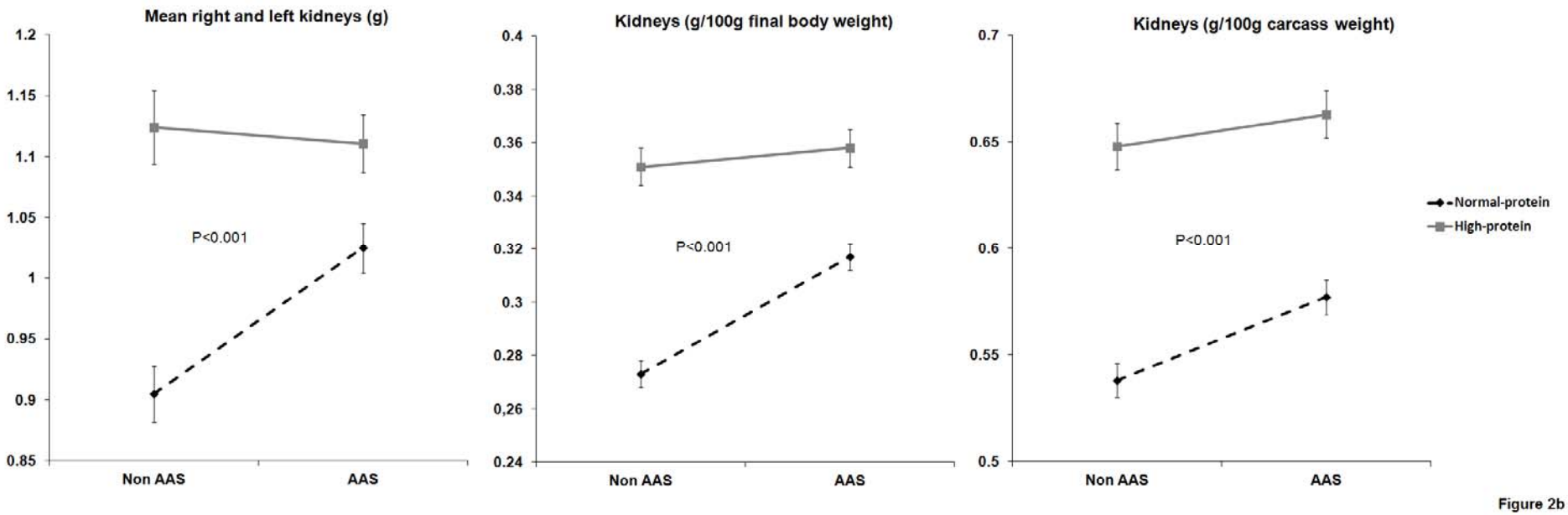

Figure 2a. Interactions taking place on kidneys weight between diet and anabolic-androgenic steroids (AAS)

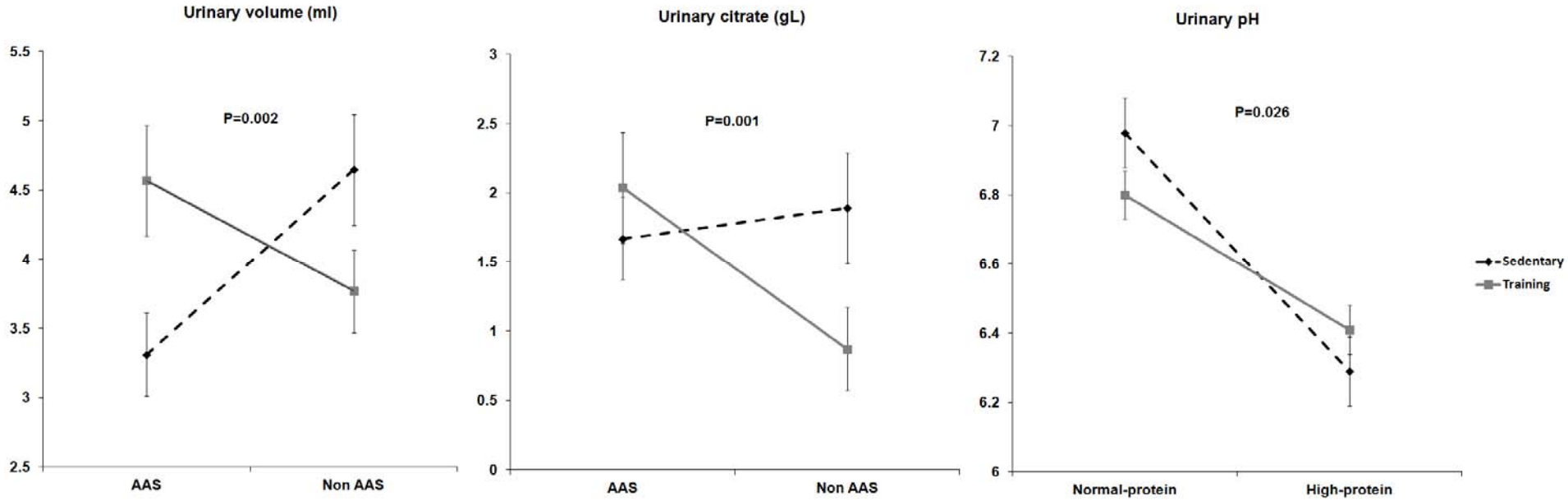

Figure 2b. Interactions taking place on urinary parameters between diet, training and anabolic-androgenic steroids (AAS)

Table 6. Interactions taking place between diet and training

Diet*Training										
L=0.719			**Normal-protein & No-Training**		**Normal-protein & Training**		**High-protein & No-Training**		**High-protein & Training**	
F(14;101)=2.8 (P<0.002)	**F**	**P**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**
Final body weight (g)	0.524	0.470	343.7 (5.4)	(332.9; 354.4)	309.6 (5.6)	(298.5; 320.7)	328.6 (5.5)	(317.7; 339.5)	296.2 (5.7)	(284.9; 307.4)
Carcass weight (g)			179.8 (2.8)	(174.2; 185.4)	168.5 (2.9)	(162.7; 174.3)	176.7 (2.9)	(170.9; 182.4)	159.8 (2.9)	(153.8; 165.7)
Plasma Urea (mg/dl)	0.035	0.852	23.9 (1.5)	(20.9; 26.8)	28.7 (1.5)	(25.7; 31.7)	31.8 (1.5)	(28.8; 34.8)	37.9 (1.5)	(34.8; 41.0)
Plasma total proteins (g/dl)	0.105	0.747	5.31 (0.09)	(5.13; 5.50)	5.55 (0.09)	(5.36; 5.74)	5.09 (0.09)	(4.90; 5.28)	5.37 (0.10)	(5.18; 5.57)
Plasma Creatine kinase (u/L)	17.460	<0.001	1424 (158)	(1111; 1737)	1036 (163)	(713; 1358)	756 (160)	(438; 1074)	1724 (166)	(1396; 2053)
Urinary Citrate (g/L)	3.048	0.084	2.91 (0.21)	(2.49; 3.33)	1.82 (0.22)	(1.38; 2.26)	0.89 (0.22)	(0.46; 1.32)	0.59 (0.22)	(0.15; 1.04)
Urinary Calcium (mg/L)	0.717	0.399	2.01 (0.13)	(1.76; 2.29)	2.10 (0.13)	(1.84; 2.35)	2.21 (0.13)	(1.96; 2.46)	2.62 (0.13)	(2.36; 2.88)
Urinary Calcium (mg/day)	0.297	0.587	0.39 (0.08)	(0.23; 0.54)	0.50 (0.08)	(0.35; 0.66)	0.64 (0.08)	(0.49; 0.80)	0.78 (0.08)	(0.62; 0.94)
Urinary volume (ml)	0.137	0.712	3.73 (0.31)	(3.11; 4.35)	3.76 (0.32)	(3.12; 4.40)	4.58 (0.32)	(3.95; 5.21)	4.39 (0.33)	(3.74; 5.04)
Urinary pH	5.074	0.026	6.98 (0.07)	(6.83; 7.12)	6.80 (0.08)	(6.65; 6.95)	6.30 (0.07)	(6.15; 6.44)	6.41 (0.08)	(6.26; 6.57)
Kidney (g) (mean R and L)	2.553	0.113	1.02 (0.02)	(0.98; 1.06)	0.94 (0.02)	(0.90; 0.99)	1.18 (0.02)	(1.14; 1.22)	1.04 (0.02)	(0.99; 1.08)
Kidney (g/100g body weight)			0.299 (0.006)	(0.287; 0.312)	0.305 (0.007)	(0.292; 0.318)	0.36 (0.006)	(0.347; 0.373)	0.351 (0.007)	(0.338; 0.364)
Kidney (g/100g carcass)			0.567 (0.009)	(0.549; 0.585)	0.561 (0.009)	(0.543; 0.579)	0.666 (0.009)	(0.647; 0.684)	0.65 (0.009)	(0.631; 0.668)
Kidney fibrosis (%)	0.899	0.345	3.02 (0.23)	(2.57; 3.47)	3.61 (0.23)	(3.15; 4.08)	2.93 (0.23)	(2.48; 3.39)	3.78 (0.24)	(3.31; 4.25)
Kidney fibrosis area (µm²)			3934 (293)	(3352; 4516)	4806 (303)	(4205; 5407)	3733 (299)	(3142; 4324)	4874 (309)	(4263; 5485)
Mesangium (%)			65.75 (1.06)	(63.66; 67.85)	63.62 (1.09)	(61.46; 65.78)	64.35 (1.07)	(62.22; 66.48)	63.44 (1.11)	(61.24; 65.63)
Mesangium area (µm²)			4837 (357)	(4130; 5544)	5722 (369)	(4991; 6452)	5609(363)	(4890; 6327)	6224 (375)	(5481; 6967)
Floculus I (%)			17.62 (1.31)	(15.02; 20.21)	22.29 (1.35)	(19.61; 24.97)	19.31 (1.33)	(16.68; 21.95)	21.78 (1.37)	(19.05; 24.50)
Floculus I area (µm²)			7699 (595)	(6520; 8878)	9168 (615)	(7951; 10386)	9022 (605)	(7824; 10220)	9884 (625)	(8646; 11123)
Floculus II (%)			37.63 (2.64)	(32.40; 42.86)	51.39 (2.72)	(45.99; 56.79)	41.16 (2.68)	(35.85; 46.48)	46.06 (2.77)	(40.57; 51.56)
Floculus II area (µm²)			16314 (1167)	(14003; 18624)	20848 (1205)	(18461; 23234)	19093 (1185)	(16746; 21441)	20715 (1226)	(18289; 23143)
Tubular fibrosis (%)			2.16 (0.18)	(1.81; 2.51)	2.92 (0.18)	(2.56; 3.28)	2.02 (0.18)	(1.66; 2.37)	2.65 (0.19)	(2.28; 3.02)
Glomerular area (µm²)	0.008	0.929	43834 (745)	(42359; 45309)	41496 (769)	(39973; 43019)	46967 (757)	(45468; 48466)	45773 (782)	(44223; 47322)

Values expressed as mean (standard error of the mean); CI, confident interval; R, right; L, left.

Table 7. Interactions taking place between diet and anabolic androgenic steroids (AAS

Diet*AAS										
L=0.696			**Normal-protein & Non-AAS**		**Normal-protein & AAS**		**High-protein & Non-AAS**		**High-protein & AAS**	
F(14;101)=3.2 (P<0.001)	F	P	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**
Final body weight (g)	0.541	0.464	332.6 (7.6)	(317.7; 347.6)	324.4 (5.4)	(313.7; 335.1)	322.4 (7.7)	(307.1; 337.7)	308.2 (5.4)	(297.4; 319.0)
Carcass weight (g)			167.2 (3.7)	(159.9; 174.6)	177.9 (2.7)	(172.6; 183.2)	174.1 (3.8)	(166.5; 181.6)	165.8 (2.7)	(160.4; 171.1)
Plasma Urea (mg/dl)	6.698	0.011	27.8 (1.9)	(24.1; 31.5)	25.4 (1.3)	(22.8; 28.1)	31.1 (1.9)	(27.3; 34.9)	36.6 (1.4)	(33.9; 39.3)
Plasma total proteins (g/dl)	0.130	0.719	5.35 (0.12)	(5.12; 5.58)	5.47 (0.08)	(5.30; 5.64)	5.20 (0.12)	(4.97; 5.44)	5.24 (0.09)	(5.07; 5.41)
Plasma Creatine kinase (u/L)	7.251	0.008	1174 (200)	(778; 1571)	1268 (143)	(984; 1552)	617 (205)	(211; 1024)	1528 (145)	(1240; 1815)
Urinary Citrate (g/L)	3.513	0.063	2.85 (0.27)	(2.31; 3.39)	2.15 (0.20)	(1.76; 2.53)	0.66 (0.28)	(0.11; 1.22)	0.79 (0.20)	(0.40; 1.18)
Urinary Calcium (mg/L)	0.387	0.535	2.28 (0.155)	(1.974; 2.586)	1.937 (0.111)	(1.718; 2.156)	2.536 (0.158)	(2.222; 2.849)	2.343 (0.112)	(2.122; 2.565)
Urinary Calcium (mg/day)	2.671	0.105	0.72 (0.09)	(0.55; 0.90)	0.30 (0.07)	(0.17; 0.43)	0.83 (0.09)	(0.65; 1.02)	0.65 (0.07)	(0.52; 0.78)
Urinary volume (ml)	0.328	0.568	3.47 (0.38)	(2.71; 4.23)	3.88 (0.27)	(3.33; 4.43)	4.48 (0.39)	(3.70; 5.26)	4.49 (0.28)	(3.94; 5.05)
Urinary pH	2.665	0.105	7.05 (0.09)	(6.87; 7.23)	6.81 (0.06)	(6.68; 6.94)	6.34 (0.09)	(6.16; 6.52)	6.36 (0.07)	(6.23; 6.49)
Kidney (g) (mean R and L)	13.577	<0.001	0.91 (0.03)	(0.85; 0.96)	1.03 (0.02)	(0.99; 1.06)	1.12 (0.03)	(1.07; 1.18)	1.10 (0.02)	(1.06; 1.14)
Kidney (g/100g body weight)			0.273 (0.007)	(0.259; 0.287)	0.317 (0.005)	(0.307; 0.327)	0.351 (0.007)	(0.337; 0.365)	0.358 (0.005)	(0.348; 0.368)
Kidney (g/100g carcass)			0.539 (0.011)	(0.518; 0.56)	0.577 (0.008)	(0.562; 0.592)	0.648 (0.011)	(0.626; 0.67)	0.663 (0.008)	(0.647; 0.678)
Kidney fibrosis (%)	0.365	0.547	3.31 (0.29)	(2.74; 3.89)	3.30 (0.21)	(2.89; 3.71)	3.49 (0.30)	(2.90; 4.07)	3.27 (0.21)	(2.86; 3.69)
Kidney fibrosis area (µm²)			4428 (378)	(3678; 5179)	4318 (271)	(3782; 4855)	4504 (388)	(3736; 5273)	4174 (275)	(3631; 4718)
Mesangium (%)			63.58 (1.30)	(61.01; 66.16)	65.30 (0.93)	(63.46; 67.14)	62.30 (1.33)	(59.66; 64.94)	64.71 (0.94)	(62.85; 66.58)
Mesangium area (µm²)			4887 (446)	(4003; 5770)	5459 (319)	(4827; 6091)	6142 (457)	(5237; 7047)	5788 (323)	(5148; 6428)
Floculus I (%)			19.31 (1.6)	(16.02; 22.60)	20.17 (1.19)	(17.81; 22.52)	21.83 (1.70)	(18.45; 25.20)	19.84 (1.20)	(17.46; 22.23)
Floculus I area (µm²)			7837 (743)	(6367; 9308)	8703 (532)	(7651; 9756)	9891 (761)	(8384; 11398)	9213 (538)	(8147; 10278)
Floculus II (%)			44.40 (3.44)	(37.58; 51.22)	44.23 (2.46)	(39.35; 49.12)	46.30 (3.53)	(39.32; 53.29)	42.15 (2.50)	(37.21; 47.09)
Floculus II area (µm²)			17847 (1484)	(14908; 20786)	18846 (1062)	(16742; 20949)	20796 (1521)	(17785; 23808)	19418 (1075)	(17289; 21548)
Tubular fibrosis (%)			2.53 (0.23)	(2.07; 2.99)	2.53 (0.16)	(2.20; 2.86)	2.24 (0.24)	(1.77; 2.72)	2.36 (0.17)	(2.03; 2.70)
Glomerular area (µm²)	0.811	0.370	40844 (913)	(39036; 42653)	43654 (654)	(42360; 44949)	45486 (936)	(43632; 47339)	46842(662)	(45531; 48152)

Values expressed as mean (standard error of the mean); CI, confident interval; R, right; L, left.

Table 8. Interactions taking place between training and anabolic androgenic steroids (AAS)

Training*AAS

L=0.611			**No-Training & Non-AAS**		**No-Training & AAS**		**Training & Non-AAS**		**Training & AAS**	
F(14;101)=4.6 (P<0.001)	F	P	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**	**mean (SEM)**	**95% CI**
Final body weight (g)	4.969	0.028	334.8 (6.6)	(321.7; 347.8)	337.0 (4.7)	(327.7; 346.3)	320.1 (6.8)	(306.7; 333.5)	294.2 (4.9)	(284.6; 303.8)
Carcass weight (g)			174.7 (3.5)	(167.7; 181.7)	180.0 (2.5)	(175.1; 185.0)	166.2 (3.6)	(159.0; 173.4)	163.2 (2.6)	(158.0; 168.3)
Plasma Urea (mg/dl)	0.097	0.756	27.0 (2.1)	(22.9; 31.0)	28.2 (1.5)	(25.3; 31.1)	32.0 (2.1)	(27.8; 36.1)	33.9 (1.5)	(30.9; 36.9)
Plasma total proteins (g/dl)	2.340	0.129	5.25 (0.12)	(5.02; 5.48)	5.18 (0.08)	(5.02; 5.34)	5.31 (0.12)	(5.08; 5.54)	5.55 (0.08)	(5.38; 5.71)
Urinary Citrate (g/L)	12.516	0.001	1123 (194)	(739; 1507)	1082 (137)	(810; 1353)	672 (199)	(278; 1065)	1735(142)	(1453; 2017)
Urinary Calcium (mg/L)	10.853	0.001	1.68 (0.31)	(1.06; 2.30)	2.04(0.22)	(1.60; 2.48)	1.89 (0.32)	(1.26; 2.53)	0.87 (0.23)	(0.42; 1.33)
Urinary Calcium (mg/day)	1.953	0.165	2.40 (0.16)	(2.09; 2.71)	1.96 (0.11)	(1.74; 2.18)	2.41 (0.16)	(2.09; 2.73)	2.33 (0.12)	(2.10; 2.55)
Plasma Creatine kinase (u/L)	1.638	0.203	0.65 (0.09)	(0.46; 0.83)	0.45 (0.07)	(0.31; 0.58)	0.91 (0.10)	(0.72; 1.10)	0.50 (0.07)	(0.36; 0.64)
Urinary volume (ml)	10.137	0.002	3.31 (0.38)	(2.56; 4.06)	4.57 (0.27)	(4.03; 5.10)	4.65 (0.39)	(3.88; 5.42)	3.77 (0.28)	(3.22; 4.32)
Urinary pH	1.506	0.222	6.79 (0.11)	(6.58; 7.01)	6.57 (0.08)	(6.42; 6.72)	6.61 (0.11)	(6.39; 6.83)	6.61 (0.08)	(6.45; 6.76)
Kidney (g) (mean R and L)	0.526	0.470	1.05 (0.029)	(0.988; 1.103)	1.125 (0.021)	(1.084; 1.165)	0.976 (0.030)	(0.917; 1.035)	0.995 (0.021)	(0.953; 1.037)
Kidney (g/100g body weight)			0.317 (0.009)	(0.298; 0.336)	0.335 (0.007)	(0.322; 0.348)	0.304 (0.010)	(0.285; 0.324)	0.339 (0.007)	(0.326; 0.353)
Kidney (g/100g carcass)			0.596 (0.015)	(0.567; 0.626)	0.625 (0.011)	(0.604; 0.646)	0.588 (0.015)	(0.557; 0.618)	0.613 (0.011)	(0.592; 0.635)
Kidney fibrosis (%)	0.035	0.851	3.00 (0.28)	(2.45; 3.56)	2.96 (0.20)	(2.57; 3.35)	3.81 (0.28)	(3.24; 4.38)	3.64 (0.21)	(3.23; 4.05)
Kidney fibrosis area (µm²)			3936 (362)	(3219; 4654)	3784 (256)	(3277; 4291)	5021(371)	(4286; 5756)	4746 (266)	(4219; 5272)
Mesangium (%)			62.83 (1.29)	(60.29; 65.38)	66.18 (0.91)	(64.38; 68.0)	63.09 (1.32)	(60.48; 65.70)	63.76 (0.94)	(61.89; 65.62)
Mesangium area (µm²)			5127 (447)	(4243; 6012)	5262 (316)	(4636; 5887)	5890(458)	(4983; 6796)	6010 (328)	(5361; 6659)
Floculus I (%)			19.13 (1.62)	(15.92; 22.34)	18.11 (1.15)	(15.84; 20.38)	22.01 (1.66)	(18.72; 25.30)	22.05 (1.19)	(19.70; 24.41)
Floculus I area (µm²)			8301 (744)	(6827; 9776)	8374 (526)	(7332; 9417)	9404(763)	(7893; 10914)	9580 (546)	(8499; 10662)
Floculus II (%)			40.78 (3.29)	(34.27; 47.30)	38.66 (2.33)	(34.06; 43.27)	50.10 (3.37)	(43.42; 56.78)	48.09 (2.42)	(43.31; 52.87)
Floculus II area (µm²)			17582 (1457)	(14697; 20467)	17731 (1030)	(15692; 19771)	21075 (1493)	(18119; 24031)	20633(1069)	(18516; 22750)
Tubular fibrosis (%)			2.04 (0.22)	(1.61; 2.48)	2.12 (0.16)	(1.81; 2.43)	2.76 (0.23)	(2.31; 3.21)	2.80 (0.16)	(2.48; 3.12)
Glomerular area (µm²)	1.205	0.275	43350 (979)	(41412; 45288)	46388 (692)	(45018; 47759)	42855 (1003)	(40869; 44841)	43979 (718)	(42557; 45401)

Values expressed as mean (standard error of the mean); CI, confident interval; R, right; L, left.

CONCLUSIONES

1. Los marcadores plasmáticos y urinarios estudiados mostraron una mayor tendencia a la acidosis metabólica tras el consumo de la dieta hiperproteica, lo que podría explicar el incremento del peso de hígado y riñón observados y favorecería el riesgo de nefrolitiasis. A pesar de esta mayor acidez, el contenido mineral óseo no se vio afectado.
2. El entrenamiento de fuerza tuvo una acción tamponadora sobre el incremento de peso de hígado y riñón ocasionado por la ingesta de dicha dieta hiperproteica.
3. El entrenamiento de fuerza incrementó el contenido mineral óseo, estimado mediante el peso en cenizas del fémur. Dicha mejora se observó especialmente a partir del segundo y tercer mes, lo que sugiere un efecto a medio-largo plazo.
4. De entre todas las intervenciones testadas (porcentaje y fuente de proteína, entrenamiento de fuerza y esteroides anabolizantes), la administración de esteroides anabolizantes fue el factor que más negativamente afectó al perfil lipídico plasmático y hepático, mientras que las dietas hiperproteicas y especialmente el entrenamiento de fuerza podrían derivar en un mejor perfil lipídico, especialmente cuando se combinan. No se han observado resultados consistentes que sustenten que el consumo de la proteína de soja sea más favorable sobre la pérdida de peso o el perfil lipídico que la proteína de lactosuero.
5. El incremento de la acidez y calciuria urinaria observados en la dieta de lactosuero podría constituir un ambiente más favorable para la formación de cálculos renales y el riesgo de enfermedades renales que la proteína de soja. Sin embargo, no se han observado diferencias morfológicas en el riñón atendiendo a la fuente de proteína.
6. Las dietas hiperproteicas y los esteroides anabolizantes aumentaron el peso del riñón y el área glomerular, especialmente cuando se combinaron. El entrenamiento de fuerza-hipertrofia redujo el peso del riñón y el área glomerular, pero ocasionó un incremento paralelo de la fibrosis renal y tubular. La alta intensidad del tipo de entrenamiento desarrollado podría ser la causa de este peor perfil histológico renal.

Conclusión general:

Los resultados de la presente memoria de Tesis ponen de manifiesto que de entre todas las intervenciones testadas (porcentaje y fuente de proteína, entrenamiento de fuerza y esteroides anabolizantes), el entrenamiento de fuerza se presentó como la mejor terapia promoviendo la pérdida de peso y mejorando, en general, el perfil lipídico hepático y plasmático. No obstante, bajo nuestra condiciones experimentales, dicho protocolo de fuerza hipertrofia indujo una menos favorable morfología renal.

CONCLUSIONS

1. Plasma and urinary markers showed higher metabolic acidity after a high whey or soy protein diet consumption, which could explain the heavier kidney and liver observed in those groups and could increase the risk of kidney stones. Despite of this acidosis, bone mineral content was not affected.
2. Resistance training was effective at enhancing bone mineral content, as measured by femur ashes weight. The effects of training were generally observed at the second and third month, suggesting a mid-long term effect.
3. Resistance training had a protective action against hepatic and renal inflammation promoted by the high protein diet.
4. Among all the interventions tested, anabolic androgenic steroids administration was the factor that most negatively affected plasma and hepatic lipid profile, whereas high-protein diets and hypertrophy resistance training could induce, in general, a better lipid profile, especially when combined. Any consistent benefits on body weight loss, hepatic and plasma lipid profile have been observed derived from soy protein instead to whey-protein consumption.
5. The increase of acid and urinary calcium excretion due to the whey-protein diet can constitute a favorable environment for kidney stones and renal diseases. However, any significant renal morphological effects attending to the protein source have been observed.
6. High-protein diets and anabolic androgenic steroids increased kidney weight and glomerular area, especially when combined. Hypertrophy resistance training reduced the higher kidney weight observed in those groups and the glomerular area, but with the parallel increase of renal and tubular fibrosis. The high intensity of the training protocol performed under our experimental design might be on the basis of this worse morphological renal status**.**

Overall conclusion:

The results of the present Thesis highlight the usefulness of resistance training among all the interventions tested (high-protein diets, whey or soy protein source, exercise and anabolic androgenic steroids) on promoting weight loss and improving plasma and hepatic lipid profile, but the present hypertrophy resistance training protocol performed under our experimental conditions induced a less favorable renal morphology.

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Actividad Académica

- **Doctora por la Universidad de Granada. Tesis Doctoral Europea: *"Condición Física, Composición Corporal y Fibromialgia"*.** Defensa 23 de septiembre de 2011. Calificación: Sobresaliente Cum Laude. Primer Premio Investigación Deportiva 2011, Consejería de Turismo, Comercio y Deporte, a través del Instituto Andaluz del Deporte.
- **Licenciada en Ciencias de la Actividad Física y del Deporte** por la Facultad de Ciencias de la Actividad Física y del Deporte de la Universidad de Extremadura. Promoción (2000-2005).
- Doctorado de "Actividad Físico-Deportiva y Calidad de Vida (950/4)", Universidad de Granada y Almería, (2005-2007).
- Máster con mención de calidad "Nutrición Humana (404/56.1)", Universidad de Granada, (2006-2007).
- Estancia de investigación en la Facultad de Ciencias del Deporte de la Universidad de Extremadura, España. Departamento de Didáctica de la Expresión Musical, Plástica y Corporal (abril-junio 2009).
- Estancias de investigación en el Karolinska Institutet. Department of Bioscience and Preventive Nutrition. Estocolmo, Suecia (15 meses en total: septiembre-diciembre 2009; mayo-noviembre 2010; marzo-julio 2011).
- Estancia de investigación en la Facultad de Ciencias de Tetuán, Marruecos (14-19 de marzo de 2010; 29 de marzo-16 de mayo de 2011).

Participación en proyectos de investigación

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3. Efectos ergogénicos y perjudiciales de los suplementos whey valorado sobre un entrenamiento de fuerza en ratas. 2007/20SVC. (2007-2010). Junta de Andalucía. Consejería de Turismo, Comercio, Ocio y Deporte. Centro Andaluz de Medicina Deportiva.
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Pósters en Congresos relacionados con la presente tesis

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