

Histological changes of liver glycogen storage in mice (*Mus musculus*) caused by high-protein diets

E. Ulusoy and B. Eren

Faculty of Arts and Science, Department of Biology, University of Ondokuz Mayıs, Samsun, Turkey

Summary. High Protein diets (HP) have been popular for people who want to lose weight since the 1960s. Even though these diets do not harm healthy people in the short term, there is insufficient data to support their safe use and efficiency over a long period. Because of the fact that the proteins in these diets are mainly from animal sources, it induces a higher intake of total fat, saturated fat and cholesterol.

It is proven that high protein diets cause both physical and pathological abnormalities in the body. However, there exist very few studies about the effects of high protein nutrition on liver glycogen storage.

For this study 40 Swiss albino mice consisting of two groups were used. The first group was fed with 25% High Protein; the other was fed with standard meal. The two groups were fed with respect to their diets for 30 days. At the end of 15th, 20th, 25th and 30th days 5 from each group were killed with cervical dislocation. The livers were removed after perfusion then fixated. The routine paraffin pursuit was applied before cutting into 5 μ m sections and staining with H-E, PAS and silver.

There were major differences in weight loss between the first and the fifteenth days. Glycogen storage was significantly reduced in HP (15) stained with PAS. Hydropic degeneration and regenerative activity was observed in H-E and silver stained HP group.

As a result for the high protein diet group, weight loss at the 15th day and a significant decrement in glycogen storage at the 30th day was observed.

Key words: Liver, High Protein Diet, Glycogen, Mice

Introduction

The liver, weighing approximately 1500g, is the largest gland in the body. It is located in the upper right-hand quadrant of the abdominal cavity, just inferior to the diaphragm and together with the gall bladder and

bile ducts it is also the most complex organ in the body (Gartner and Hiatt, 1997; Kumar et al., 2000). Besides secretion it has many other essential functions. Hepatocyte cells are the main parts that materialize these functions and form the major part of liver. A single liver cell can carry out more than 500 separate, specialized metabolic activities (Solomon et al., 1999). Among these specialized activities the most significant one is glycogen storage. The decrease or increase of glycogen storage of the liver is related to the diet of the subject. (Andreoli et al., 2000).

High-protein diets are not recommended because they restrict healthy foods that provide essential nutrients and do not provide the variety of foods needed to adequately meet nutritional needs. Individuals who follow these diets are therefore at risk of compromised vitamin and mineral intake, as well as potential cardiac, renal, bone, and liver abnormalities overall (Jeor et al., 2001). However, there exist very few studies about the effects of high protein nutrition on liver glycogen storage.

American Heart Association (AHA) Nutrition Committee suggests that high protein intake may have detrimental effects on liver function. However, until now there has not been sufficient scientific evidence supporting this contention. Protein is needed not only to promote liver tissue repair, but also to provide lipotropic agents such as methionine and choline for the conversion of fats to lipoprotein and to be removed from the liver, thus preventing fatty infiltration (Navder and Lieber, 2003).

It is possible to find many studies based on the effects of unbalanced diets on the metabolism. The amount of protein used in high protein diets exceeds the need of the body and may impose significant health risks. In high protein diets Animal based proteins are preferred rather than plant based proteins that also contain carbohydrates. A diet rich in animal protein, saturated fat and cholesterol raises low density lipoprotein (LDL) cholesterol levels. On the other hand high carbohydrate, high-fiber plant diets limits or eliminates LDL (Larosa et al., 1980; McDowell et al., 1991.)

Furthermore, a high-carbohydrate diet that includes

fruit, vegetables, non-fat dairy products, and whole grains has been shown to lower blood pressure (Appel et al., 1997). In other words limitation of these foods may raise blood pressure and may cause reduction in potassium, calcium, and magnesium coupled with an increase in sodium intake. High protein foods such as meat, poultry, seafood, eggs, seeds, and nuts are high in purines. Purines are broken down into uric acid, so excess consumption of these foods increases uric acid levels (Franzese, 2000). A surplus of protein in the system also increases urinary calcium loss, which may increase the risk of osteoporosis (Abelow et al., 1992; Feskanich et al., 1996; Barzel and Massey, 1998). High Protein Diets increase nitrogen in the tissues; as a result it stimulates amino acid degradation, followed by a large rise in urea production in liver (Morens et al., 2000). As a result, increased glomerular filtration rates (Hammond and Janes, 1998), renal and glomerular hypertrophy (Schrijvers et al., 2002), and decreased urine pH. (Schuette, 1980; Fellstrom et al., 1983) were observed. Increased uric acid levels may cause gout in sensitive individuals (Abelow et al., 1992; Franzese, 2000). In addition a decrease in calcium by urinating may increase the risk of kidney stones (Wiederkehr and Krapf, 2001; Reddy et al., 2002) also some types of cancer (Willet et al., 1990; Giovannucci et al., 1994).

Moreover there has been research on mental performance of individuals and it was observed that HP diets lead to lower memory performance and lower level of concentration. These results suggest that carbohydrate and protein based diets have different effects on mental health. For example, Smith et al. (1988) compared the performance of three groups which have different diets (high-starch, high-sugar, or high-protein) on concentration and reported that the carbohydrate diet group has better performance than the others (Dye et al., 2000).

Also Jorda et al. (1988) reported that the liver responds to the high-protein diet by a proliferation of normally functioning mitochondria.

Apart from this histologic research there has not been another on the effects on liver glycogen storage on the metabolism those who are on high protein diets. For this reason the aim of this research is to identify histologically the change dependence at term in the liver glycogen storage of the mice having HP diets.

Materials and methods

Forty male Swiss albino laboratory mice (*Mus musculus*), 60-90 day-old, average initial body weights 24-42 g, were used in this study. The animals were housed in individual wireless steel cages under standardized conditions of temperature and light.

The animals were divided into two groups. The first group was the one which was fed with HP diets (casein) the other was the control (C) group that was fed with standard diet. All the mice were given 12 ± 2 g diet per day in separated cages. While the control group was given a standard diet the other was fed with 25% HP diet

(Reeves et al., 1993). HP diet used on mice was prepared by powder (Table 1). This powder has made by fat-free casein and gluten-free. As 2.5 g powder was equal to 2.2 g protein; this 25% HP was prepared, diluting distilled water $6.3 \pm 1 + 2.5$ g powder.

The animals were free access to water and were fed with their own diet for 30 days. At the end of 15th, 20th, 25th and 30th days, 5 from each group were killed by cervical dislocation after being weighed. The livers were perfused with saline solution (0,9%) injected through main hepatic artery and then removed. Later the liver tissues were fixated with 80% ethanol for 12 hours. The fixated liver tissues were embedded in paraffin and cut into 5 μ m sections and then stained with H-E, PAS and silver (Bancroft and Stevens, 1996).

In our study, we used analytically pure substances provided by Sigma-London Company, standard meal was provided by Samsun Yem Company and the protein which was used to prepare the HP diet was provided by Nutricia Cuijk-Holland Company.

Data gathered was examined in five different periods for normal distribution. For data a normal distribution analysis of variance (ANOVA) was used. Statistics were performed by the statistical package SPSS for Windows. The limit of significance was set at $p < 0.05$.

Results

In the high protein group weight loss was observed in the mice of 15-25 days when compared to the 1st day and also to the control group. The weight loss between the 1st day and the 15th day was meaningful ($P < 0.05$). However, there was statistically no weight difference between 15th day and the 30th day ($P > 0.05$). There was not a significant weight decrease or increase in the control group (Fig. 1). It was observed that the livers acquired from the HP group were darker (Fig. 2).

PAS technique allowed us to observe a significant decrease in glycogen storage on 15th day of HP group. Firstly, it was observed that glycogen deposits in 15 th day in control group (Figs. 3, 4). Glycogen was found in minimum amounts around central vein and periportal areas. This amount decreased from 15th day (Figs 5, 6)

Table 1. Average analysis per 100 g.

Energy	1570kJ/370kcal
Protein (95,6 En%)	88,5g
Carbohydrate (0,5En%)	
Of which sugars (lactose)	≥ 1 g
Fat (3,9En%)	≥ 2 g
Fibre	-g
Mineraller	
Na	<40mg
K	<90mg
Ca	1350mg
P	700mg
Mg	<30mg
Cl	<120mg

Effects of high protein diet on liver

to 30th. On the 30th day (Fig. 7) the glycogen storage was almost finished. A small amount of dilatation in sinusoids was detected (Fig. 8).

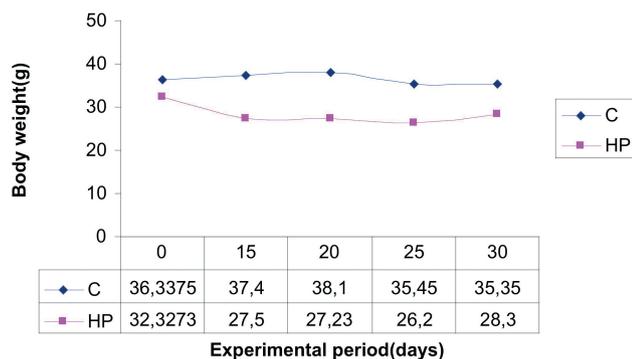


Fig. 1. Weight curves of mice, fed high protein (HP) and normal(C) diets. Body weights of 30 day old mice.

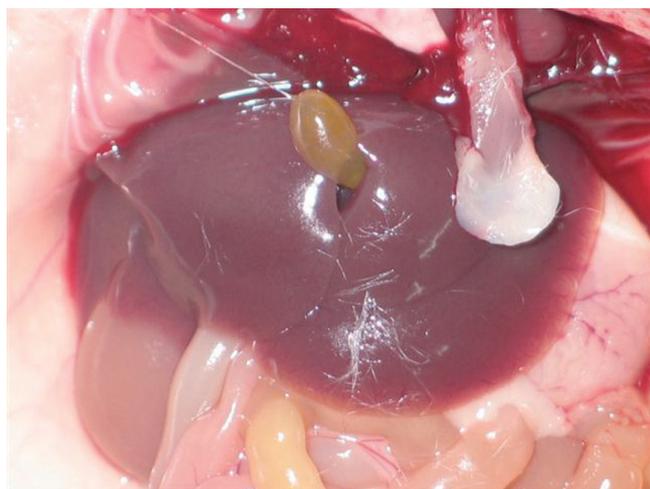


Fig. 2. A. The macroscopic view of the control group livers of the mice. **B.** Showing a remarkable macroscopic view of the mice which were fed with High-protein diet were darker).

In H-E staining no difference on radial symmetry in hepatocyte of HP liver tissues was detected and portal triads were observed as normal and also hydropic degeneration was on a small-scale (Fig. 9).

It was observed that regenerative activity was mainly in 25th and 30th day in HP group stained with silver method (Fig. 10).

Discussion

The explanation of the first weeks of HP diet group's loss of weight (12.5%) and the stability afterwards is as follows: It is known that mobilization of protein stores supply amino acids for liver gluconeogenesis in many

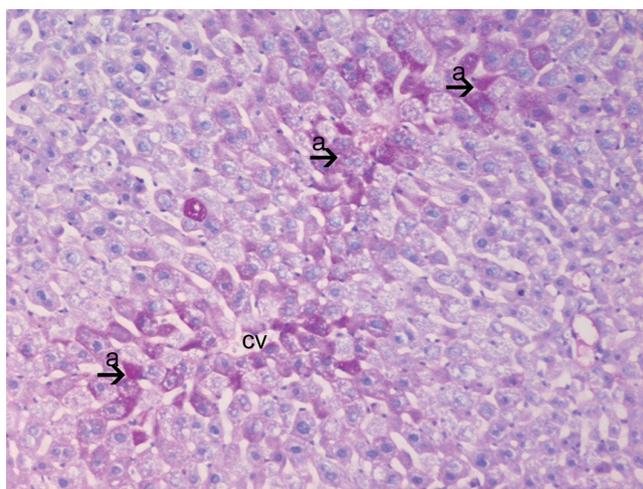


Fig. 3. Light micrograph of the histological view of livers in C (15). Observe densely glycogen deposits in the hepatocyte cytoplasm (a) around the central vein (cv). These accumulations are seen as pink areas of PAS-positive material throughout the section. PAS, x 200

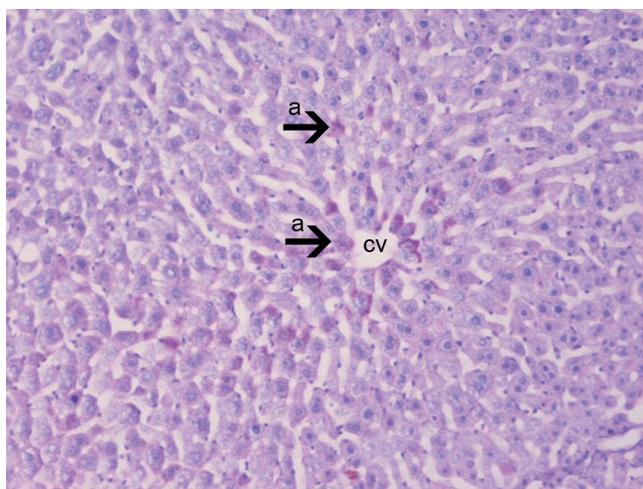


Fig. 4. Light micrograph of the histological view of livers in C (15). The glycogen deposits were removed leaving only the plasma membrane and certain carbohydrate (a) staining pink. PAS stain after diastase digestion, x 200

vertebrates in the absence of food intake (Kraus-Friedmann, 1984). The usage of this metabolic pathway increases in HP diets and remains constant on lack of long-term food intake (Silva and Migliorini, 1990; Sartori et al., 1995). HP diets promise that as long as carbohydrates are restricted, some weight will be lost; despite no limitation in food consumption. High protein and high lipid diets start metabolic ketosis, and these diets are charming because they lead to rapid loss of

weight. The weight loss in the short term was reported to be a result of the diuretic effect that was caused by low carbohydrate intake and it was proved that this low calorie intake might cause lack of appetite as long as the same diet was kept up (Jama, 1973; Denke, 2001). Some high protein, very low carbohydrate, weight-loss diets induce ketosis and when carbohydrate intake or utilization is insufficient to provide glucose to the cells, ketone bodies that are formed from fatty acids are used as an energy source. An increase in ketones can disturb the body's acid-base balance, causing metabolic acidosis

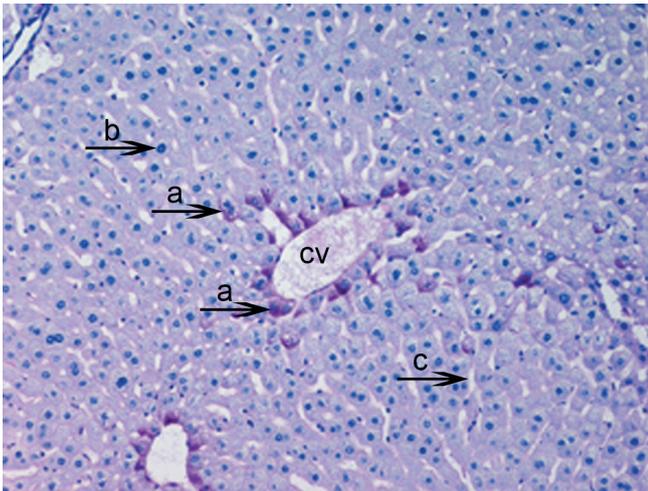


Fig. 5. Light micrograph of the histological view of livers in HP (20). Observe the minimal quantities of glycogen deposits in the hepatocyte cytoplasm (a) around the central vein (cv). They correspond to the pink-purple stained areas. The nucleus of hepatocyte (b) and sinusoids (c). PAS, x 200

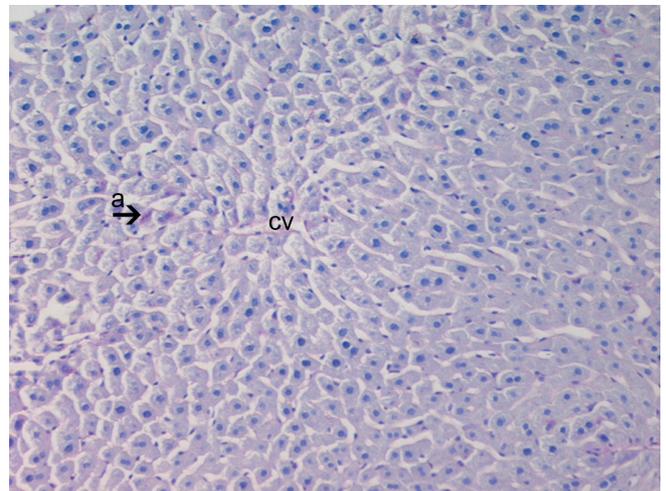


Fig. 6. Light micrograph of the histological view of livers in HP (20). Observe the similarity of the image as in figure 5. The glycogen deposits were removed and only minimal quantities of certain carbohydrate were left (a). PAS stain after diastase digestion, x 200

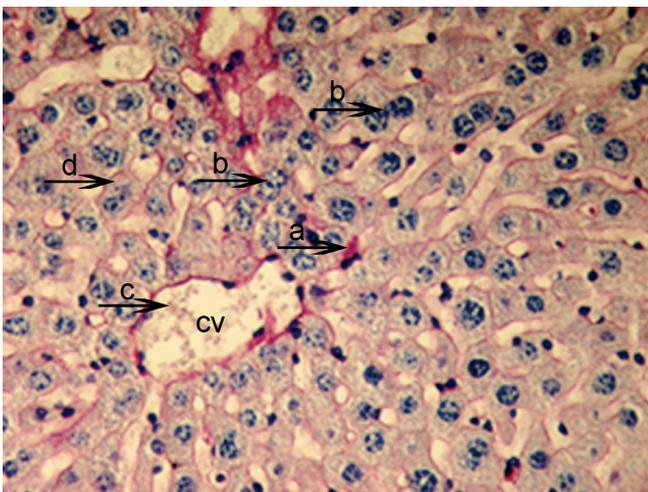


Fig. 7. Light micrograph of the histological view of livers in HP group (30). Glycogen deposits decreased to a minimum (a), showing a remarkable increase of regenerative activity of hepatocytes (b), erythrocytes (c), brownish deposits within hepatocytes (d) and central vein (cv). PAS, x 600

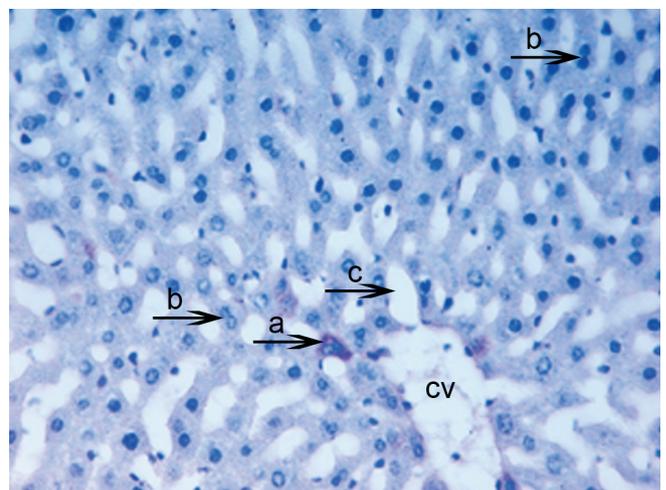


Fig. 8. Light micrograph of the histological view of livers in HP group (15). Observe the little amount of glycogen deposits (a) around the central vein (cv). Binucleate liver cells (b), little width sinusoids (c). PAS, x 400

Effects of high protein diet on liver

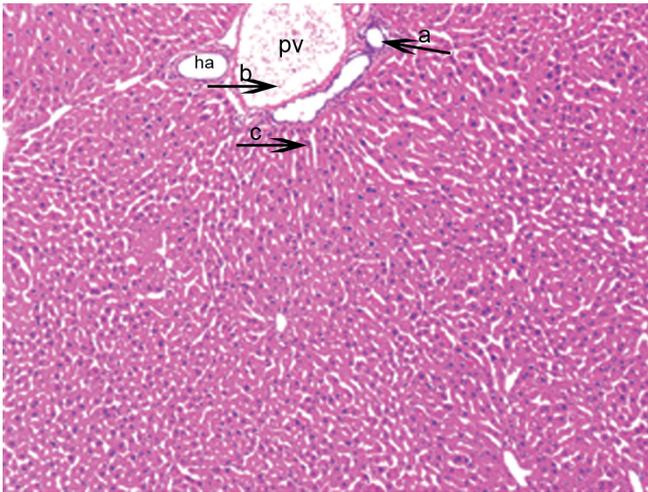


Fig. 9. Light micrograph of the histological view of livers in HP group (15). Also, a portal canal appears with normal aspect. Portal vein (pv), hepatic artery (ha), bile duct (a) and erythrocytes. Hepatocytes (c) appear as interconnecting cords. H-E x 150

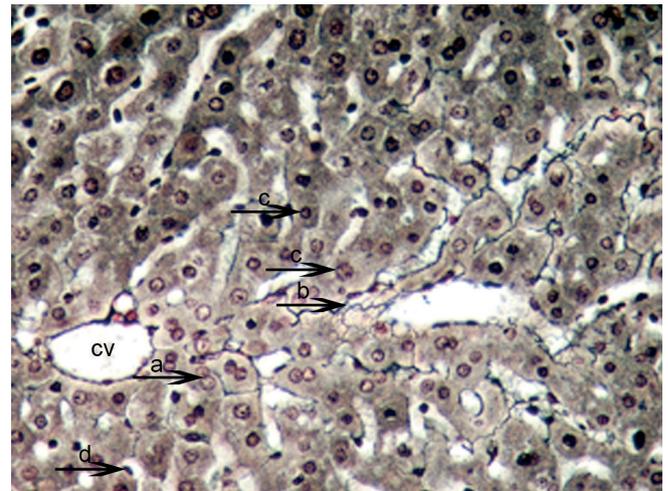


Fig. 10. Light micrograph of the histological view of livers in HP group (25). Showing a remarkable increase in regenerative activity (a) around the central vein (cv), reticular fibers (b), the differences between nuclei size (c) and The Kupffer cells (d). Silver, X 600.

(Wiederkehr and Krapf, 2001). The weight loss in the HP group has the same characteristics as the weight losses in the studies mentioned above.

It was observed that the livers of the animals which were fed with HP were darker than the control group's. Kumar (2000) has declared that the reasons for visible protein deposition are intake of protein to cells or excess synthesis of protein in cells. According to the results of this study, it can be declared that the colour change was due to excessive protein storage in hepatocytes. Histological examination proved that the glycogen storage in HP group kept decreasing through the 15th till the 30th day.

Insulin resistance can lead to a reduction in glycogen synthesis (Radziuk, 1982) whereas elevation in the catecholamine levels can contribute to increased glycogen degradation (Kuwajima et al., 1986; Fedatto-Junior et al., 2002). In addition to this result explaining the glycogen decrease; gluconeogenesis way is known to require much more energy (Nelson and Cox, 2000).

According to Kumar (2000) an unbalanced diet is one of the main causes of cell damage. He reported that after rapid consumption of glycogen, glycolysis increased and the hydrolysis of phosphate esters allows lactic acid and inorganic phosphate to accumulate. He also added that this condition caused a decrease of pH in cells. Reports support dilatations in sinusoids and hydropic degenerations found in this research.

In our studies a significant regenerative activity in HP diet groups was observed, compared to control groups. Research done by Jorda et al. (1988) supports our findings. According to the research, rodents fed very high protein intakes have been found to exhibit morphological changes in the liver mitochondria, which could be pathological. Moreover, the branched-chain amino acids to aromatic amino acids ratio were also

increased, indicating the absence of hepatic failure in these animals. The increased protein content of diet induced rapid increases in several characteristics of hepatocytes. The results presented here constitute a good example of how the hepatocyte adapts to a continuing metabolic stress (Manninen, 2004). Our observation of increased regenerative activity is supported by this condition.

As a result, energy loss with weight lost in our high-in-protein low-in-carbohydrate group is observed and histologically it is seen that glycogen storage had been reduced simultaneously. Our study actually pointed out that weight loss corresponds to water and glycogen loss, which happens to be unhealthy. It supported our belief that mice used gluconeogenesis way to produce energy but it was not sufficient for the body and as long as this gluconeogenesis way kept on going we could understand that the liver might easily be damaged (vacuolation).

Further, protein catabolism is increased in liver disease and may be exacerbated by inadequate protein in the diet. Unless there is encephalopathy (vide infra), the diet should provide high-quality protein in the amount of 1,5 to 2 g/kg (Navder and Lieber, 2003). In alcoholic liver disease, a high calorie, high-protein diet has shown to improve hepatic function and reduce mortality. In one study, this was achieved by providing a regular diet plus supplements of 60 g/day of protein and 1600 kcal/day for the first 30 days and followed by supplements of 45 g/day of protein and 1200 kcal/day for the next 60 days (Mendellhall et al., 1993). Taking these results into consideration necessary arrangements in our diets should be made.

The results show that unbalanced diets can cause noteworthy damage in liver as well as any other tissue of the organism; getting help from a professional for the ingredients of a daily diet is undoubtedly a necessity.

References

- Abelow B.J., Holford T.R. and Insogna K.L. (1992). Cross-cultural association between dietary animal protein and hip fracture: a hypothesis. *Calcif. Tissue Int.* 50, 14-18.
- Appel L.J., Moore T.J. and Obarzanek E. (1997). For the DASH Collaborative Research Group. A clinical trial of the effects of dietary patterns on blood pressure. *N. Engl. J. Med.* 336, 1117-24.
- Andreoli T.E., Carpenter C.C.J., Griggs R. and Loscalzo J. (2000). *Cecil essentials of medicine*. 5th ed. W.B. Saunders. Chapter 7.
- Bancroft J.D. and Stevens A. (1996). *Theory and practice of histological techniques*. 4th ed. Churchill Livingstone Medical Division of Pearson Professional Ltd. pp 136.
- Barzel U.S. and Massey L.K. (1998). Excess dietary protein can adversely affect bone. *J. Nutr.* 28, 1051-1053.
- Denke M.A. (2001). Metabolic effects of high-protein, low-carbohydrate diets. *Am. J. Cardio.* 88, 586-587.
- Dye L., Luch A. and Blundell J.E. (2000). Macronutrient and mental performance. *J. Nutr.* 16, 1021-1034.
- Fedatto-Junior Z., Ishii-Iwamoto E.L., Caparroz-Assef S.M., Vicentini G.E., Bracht A. and Kelmer-Bracht A.M. (2002). Glycogen levels and glycogen catabolism in livers from arthritic rats. *Mol. Cell. Biochem.* 229, 1-7.
- Fellstrom B., Danielson B.G., Karlstrom B., Lithell H., Ljunghall S. and Vessby B. (1983). The influence of a high dietary intake of purine-rich animal protein on urinary urate excretion and super saturation in renal stone disease. *Clin. Sci.* 64, 399-405.
- Feskanich D., Willett W.C., Stampfer M.J. and Colditz G.A. (1996). Protein consumption and bone fractures in women. *Am. J. Epid.* 143, 472-479.
- Franzese T.A. (2000). Medical nutrition therapy for rheumatic disorders. In: Krause's Food, Nutrition & Diet Therapy. 10th ed. Mahan L.K., Escott-Stump S. (eds). W.B. Saunders. Philadelphia. pp 970-986.
- Gartner L.P. and Hiatt J.L. (1997). *Color textbook of histology*. W.B. Saunders Company. Philadelphia. pp 346-355.
- Giovannucci E., Rimm E.B., Stampfer M.J., Colditz G.A., Ascherio A. and Willett W.C. (1994). Intake of fat, meat, and fiber in relation to risk of colon cancer in men. *Cancer Res.* 54, 2390-2397.
- Hammond K.A. and Janes D.N. (1998). The effect of increased protein intake on kidney size and function. *J. Exper. Bio.* 201, 2081-2090.
- JAMA (1973). Statement of the American Medical Association Council on Foods and Nutrition. A critique of low-carbohydrate ketogenic weight reduction regimens. A review of Dr. Atkins' Diet Revolution. *J. Am. Med. Rec. Assoc.* 224, 1415-1419.
- Jeor S.T.S., Howard B.V., Prewitt E., Bovee V., Bazzarre T. and Eckel R.H. (2001). Dietary protein and weight reduction A statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. *Am. Heart. Assoc.* 104, 1869-1874.
- Jorda A., Zaragosa R. and Manuel P. (1988). Long-term high-protein diet induces biochemical and ultrastructural changes in rat liver mitochondria. *Arch. Biochem. Biophys.* 265, 241-248.
- Kraus-Friedmann N. (1984). Hormonal regulation of hepatic gluconeogenesis. *Physiol. Rev.* 64, 170-220.
- Kumar V., Cotran R.S. and Robbins S.L. (2000). *Basic pathology*. W.D. Saunders Company. Chap. 1-16.
- Kuwajima M., Newgard C.B., Foster D.W. and McGarry I.D. (1986). The glucose phosphorylating capacity of liver as measured by three independent assays. *J. Biol. Chem.* 261, 8849-8853.
- Larosa J.C., Fry A.G. and Muesing R. (1980). Effects of high-protein, low carbohydrate dieting on plasma lipoproteins and body weight. *J. Am. Diet. Assoc.* 77, 264 -270.
- Manninen A.H. (2004). High - protein weight loss diets and purported adverse effects: Where is the evidence? *J. Sport Rev. Nutr.* 1, 45-51.
- Mendellhall C., Moritz T. and Roselle G.A. (1993). A study of oral nutrition support with oxadrolone in malnourished patients with alcoholic hepatitis: results of a Department of Veterans Affairs Cooperative Study. *Hepatology* 17, 564-576.
- McDowell M., Briefel R. and Alaimo K. (1991). Reserved energy and macronutrient intakes of persons ages 2 months and over in the U.S: Third National Health and Nutrition Examination Survey, Phase 1, 1988-91. CDC publication No. 255.
- Morens C., Gaudichoni C., Metzges C.C., Fromentin G., Baglieri A., Even P.C., Huneau J.F. and Torne D. (2000). A high-protein meal exceeds anabolic and catabolic capacities in rats adapted to a normal protein diet. *J. Nutr.* 130,9; Healthy Module pp 2312.
- Navder K.P. and Lieber C.S. (2003). Nutritional support in chronic disease of the gastrointestinal tract and the liver. In: *Nutritional aspects and clinical management of chronic disorders and diseases*. Bronner F. (ed). CRC press. Boca Raton, FL. pp 45-68.
- Nelson D.L. and Cox M.M. (2000). *Lehninger principles of biochemistry*. 3th Edition, Chapter 20. Worth Publishers, Inc.
- Radziuk J. (1982). Sources of carbon in hepatic glycogen synthesis during absorption of an oral glucose load in humans. *Fed. Proc.* 41, 110-116.
- Reddy S.T., Wang C.Y., Sakhaee K., Brinkley L. and Pak C.Y. (2002). Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism. *Am. J. Kid. Dis.* 40, 265-274.
- Reeves P.G., Nulsen F.H. and Fahey Jr. G.C. (1993). AIN-93, Purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc Writing Committee on the reformulation of the AIN-76, a rodent diet. *J. Nutr.* 123, 1939-1951.
- Sartori D.R., Migliorini R.H., Veiga J.A., Moura J.L., Kettelhut I.C. and Linder C. (1995). Metabolic adaptations induced by long-term fasting in quails. *Comp. Biochem. Physiol.* 111A, 487-493.
- Schrijvers B.F., Rasch R., Tilton R.G. and Flyvbjerg A. (2002). High protein-induced glomerular hypertrophy is vascular endothelial growth factor-dependent. *Kid. Int.* 61, 1600-1604.
- Schuette S.A. (1980). Studies of the mechanism of protein induced hypercalciuria in older men and women. *J. Nutr.* 110, 305-315.
- Silva S.M. and Migliorini R.H. (1990). Effects of starvation and refeeding on energy-linked metabolic process in the turtle. *Comp. Biochem. Physiol.* 96A, 415-419.
- Smith A.P., Leekham S., Ralph A. and McNeill G. (1988). The influence of meal composition on post-lunch performance efficiency and mood. *Appetite* 10, 195-203.
- Solomon E.P., Berg L.R. and Martin D.W. (1999). *Biology*. fifth edition. Saunders College Publishing. Chapter 45.
- Wiederkehr M. and Krapf R. (2001). Metabolic and endocrine effects of metabolic acidosis in humans. *Swiss Med. Wkly.* 131, 127-132.
- Willett W.C., Stampfer M.J., Colditz G.A., Rosner B.A. and Speizer F.E. (1990). Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. *N. Engl. J. Med.* 323, 1664-1672.