Acta Veterinaria (Beograd), Vol. 43, No. 2—3, 77— 86, 1993.

UDK 619:612.397.81

EXPERIMENTAL HYPERLIPOPROTEINEMIA IN RABBITS INDUCED BY DIETS CONTAINING CHOLESTEROL OR SUCROSE

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(Received, 19 Januar 1993)

The aim of the study was to compare the hyperlipoproteinemia occuring after a sucrose containing diet to the hyperlipoproteinemia occuring after a cholesterol containing diet.

Rabbits were kept on dietary regimes in the same period as a group of rabbits on a normal diet showed aberrations from normal lipoprotein values. Each dietary regime induced hyperlipoproteinemia of a different type.

Morphological changes induces by the experimental diets were the most severe on the blood vessels. With the cholesterol diet, changes in the aorta as well as in vasa vasorum dominated, while the sucrose diet resulted in lesions in the corany vessels, pulmonary and adrenal arteries.

Key words: Rabbits, cholesterol diet, sucrose diet, hyperlipoproteinemia

INTRODUCTION

Hyperlipidemia may be manifested by an increase of cholesterol only, triglycerides only, or an icrease of both, wich is associated with an increase of one or more lipoprotein fractions. However, it has also been reported that an increase of lipoprotein levels may be accompanied by normal lipid levels (Albers et al., 1978).

Changes in feed ingredients may have different influences on plasma lipids and lipoprotein levels. This includes alterations in both quantitative and qualitative exogenous lipid contents. A secondary effect influences the synthesis of cholesterol and lipoproteins, triglycerides, or changes the secretion of biliary acids, intestinal cholesterol and its metabolism (Ramesha et al., 1980).

The serum lipoproteins of rabbits fed with casein or soya proteins differ greatly. The lipoprotein spectrum of rabbits fed with soya proteins with the addition of lysine is similar to that of rabbits fed with casein (Czarnicki and Kritchevsky, 1979).

A protein-rich diet is generally much more atherogenic than a lowprotein diet. Animal proteins are, generally, far more atherogenic but the effect may depend on the type of carbohydrates or proteins in the diet. The effect of carbohydrates may be significantly influenced by an addition of 0.1% cholesterol

to the diet. Nevertheless, a particular combination of feed components (if the mixture in the diet is neglected) need not necessarily yield the expected results.eg. milk contains casein, cholesterol, saturated fats, and is still not hypercholesterolemic for humans (Mann, 1977; Howard and Marks, 1977) or rats (Nair and Mann, 1977; Kritchevsky et al., 1979).

Oils of different origin have different atherogenic effects. Thus, fish oil in the diet reduces serum cholesterol values and inhibits atherosclerosis. Morover, HDL-cholesterol values are lower compared to a diet with pure coconut oil (Davis et al., 1987). Sunflower oil in diets for rats gave significant elevations of plasma triglycerides, total cholesterol, HDL-cholesterol, hepatic cholesterol, fatty acid synthesis and hepatic low density lipoproteins (LDL) as well as their receptor activity compared with rats fed with fish oil. These authors conclude that fish oil promotes cholesterol metabolism in rats including, therefore, mutual changes in activitaes of hepatic LDL and HDL receptors (Roach et al., 1987).

A fish oil diet plays a protective role and controls the advance of atherosclerosis in the course of severe atherogenic stimuli (Davis et al., 1987).

It has been suggested that immunological mechanisms participate in atherogenic processes since immunoglobulins are frequently present in atherogenic plaques in the aorta. The available data also suggest that the presence of normal antibodies for modification of LDL influences a shift such as transfer of LDL from plasma into reticuloid liver cells. Thus, antibodies might have a protective role (Wiklund et al., 1987).

Very interesting findings have been reported on the stimulation of lipoprotein lipase by a cholesterol containing diet.Macrophages inhibited by VLDL-lipoproteins obtained from rats fed normally secreted twice as much lipoprotein lipase as those without additional lipoproteins. Low and very low density lipoproteins of rats fed a cholesterol-rich diet also indirectly promoted secretion of lipoprotein lipase. Lipoproteins stimulating secretion of lipoprotein lipase induced intracellular accumulation of both triglycerides and cholesterol (Mori et al., 1987).

Production of very low density lipoproteins in perfused liver was also studied in non-human primates receiving normal and cholesterol- rich diets. These data illustrate two importand features of VLDL production. First, VLDL are secreted from the liver in a form similar to that found in plasma. Therefore, in normally fed animals VLDL from plasma and perfused liver have similar lipid contents. Further, in non-human primates, VLDL composition may be significantly modified by a diet which differs from the normal one.

The nature of dietary fats plays an important role in the determination of the profile and composition of lipoprotein and its secretion in the liver of primates. Production of density lipoproteins has been investigated,which probably arive in the plasma from different sources, and which may have a possible role in the secretion of lipid poor apoplipoproteins A-I and A-II (Getzl et al., 1987).

Dietary starch, in comparison to sucrose, produces cholesterol enriched medium density lipoproteins and leads to a rise of low density lipoproteins, while sucrose leads to an increase of high density lipoproteins which are partly

phospholipids, cholesterol and apoliproteins A-I, while triglycerides enrich very low density lipoproteins (Srinivasan et al., 1987).

Some investigations have shown that a combined diet including carbohydrates, lipids and proteins is better than a (fiber) natural diet for obtaining a response in serum and liver lipids. Statistical analysis has indicated that desirable lipid responses may be obtained with a diet containing: 3—5% of ordinary bran, 6—10% of lipids, 54—55% of carbohydrates, 26—30% of proteins and 4.7% of vitamins and minerals (Stewar et al., 1987). It was concluded that the relative ratio of carbohydrates from starch and sucrose influences the size of the lipoprotein rensponse in cholesterol containing diets (Srinivasan et al., 1988).

More recent studies focused on the dietary effect of olive oil in rats and reported significantly lower levels of high density lipoproteins, cholesterol and apolipoproteins, concurrently with significantly higher liver triglyceride content as compared to rats fed with oleic-sunflower oil or linoleic-sunflower oil. This suggests that HDL and lipid contents in the liver have been determined by some features of the dietary oils apart from the incorporated oleic acid (Ney et al., 1989).

The effect of the same synthetic diet on different strains of the same animal species concemed liability to atherosclerosis. The authors concluded that HDL in species liable to atherosclerosis have a lower capacity for cholesterol transport, i. e. that HDL particles are significantly smaller in liable species than in species resistant to atherosclerosis (Nishina et al., 1990).

Palm oil in cholesterol-free diets need not necessarily contribute to the development of coronary heart diseases, since both palmitic and stearic acids are incorporated primarily in the liver and adipose tissue, and only to a minor degree in the plasma. This is important because of the decreased capacity for deposition in the arterial walles as well as because of antithrombotic properties of palm oil (Pereira et al., 1990).

Oxidative modification of lipoproteins is important for inducing arteriosclerosis. Application of antioxidants may have a protective effect, although the influence of antioxidants on immunological factors cannot be ruled out, either (Bjorkhem et al., 1991).

Thus investigations related to the effect of vitamin C on the serum cholesterol and lipoprotein levels are particularly interesting. A cholesterol diet given to in rats suggests that vitamin C deficiency slows down LDL metabolism inducing hypercholesterolemia (Uchida et al., 1990).

Oxidised low density lipoproteins (LDL) are far more atherogenic than native LDL. The initial step in oxidation is peroxidation of polyunsaturated fatty acids. A decrease of the concentration of polyunsaturated fatty acids reduces the sensitivity of LDL to oxidation. Therefore, the possibility of applying an oleate enriched diet reducing the liability of LDL to oxidative modification has been investigated thus avoiding an increase of atherogenic LDL cholesterol (Reaven et al., 1991).

MATERIAL AND METHODS

The experiment was conducted on chinchilla rabbits, of both sexes and initial body weight 1500 g.

The first experimental group was fed with a cholesterol supplement. Every day cholesterol dissolved in edible oil was instilled into the stomach through a probe 0.006 g of cholesterol per kg BW was applied for 30, 60 and 90 days. The second group was also fed through a probe using 2.5 g of sucrose dissolved in water per kg BW for 30, 60 and 90 days during the same interval.

A group of rabbits receiving food normal for the species served as the control for both experimental groups.

At the end of each dietary period the lipoproteins of experimental and control animals were determined.

Electrophoresis of serum lipoproteins (1 nl) was performend using a Pol-E-Film System.

Tissue samples for pathohistological analyses were fixed in 10% formalin, embedded in paraffin and stained by hematoxyl ineosin (HE) and then using the trichrome method of Masson and special staining for fats (Sudan III).

RESULTS

The results obtained for the experimental group of rabbits were compared to the values obtained in rabbits received normal food. The histogram in Figure 1 shows that after only a month of supplementary cholesterol the beta fraction was significantly elevated, while the alpha fraction showed a steep fall. The pre-beta fraction was also increased after a month on the cholesterol after two and three months of extra cholesterol in the diet, although it was significantly less prominent, while the pre-beta fraction showed a kind of stagnation compared to the controls (Figure 1 A).

However changes in the studied parameters in the rabbits fed sucrose, were the most prominent after two months on the diet. A significant elevation of the beta fraction was noted, while the alpha fraction was significantly reduced.

The pre-beta fraction failed to show any significant difference from the controls.

However, after the first and third month of the sucrose containing diet a mild rise of beta and pre-beta fractions was noted as compared to normally fed controls. The alpha-fraction showed a moderate decrease compared to the controls, but the fall was less prominent than that recorded after the second month on the diet (Figure 1 B).

Already after the first month the experimental group of rabbits receiving cholesterol showed severe hyperlipidemia which could be compared to hyperlipoproteinemia type III in humans.

At the end of the second month hyperlipoproteinemia i. e. hypercholesterolemia similar to type I-A in humans, characterized with a rise of lipoproteins and cholesterol was recorded in rabbits fed the extra carbohydrate (Figure 2).

Both dietary regimes led to changes in the blood vessels of organs and tissues. With the sucrose diet the alterations suggested hyalinosis of the vessels, as well as fibrous thickening of the intima of the aorta, coronary arteries and

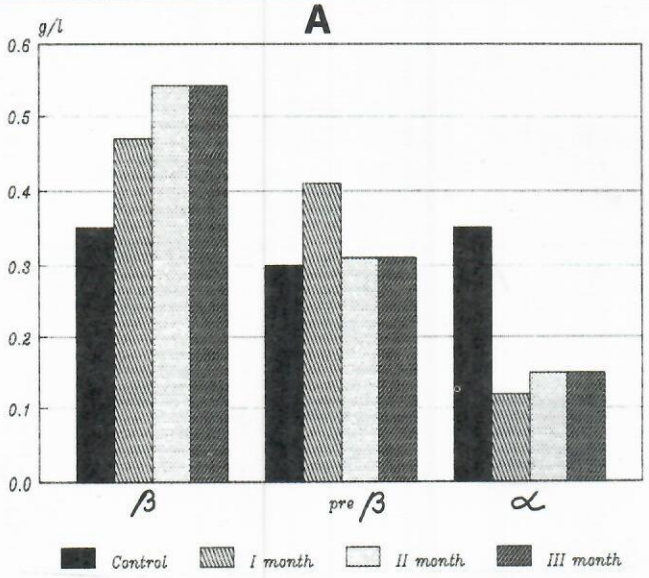
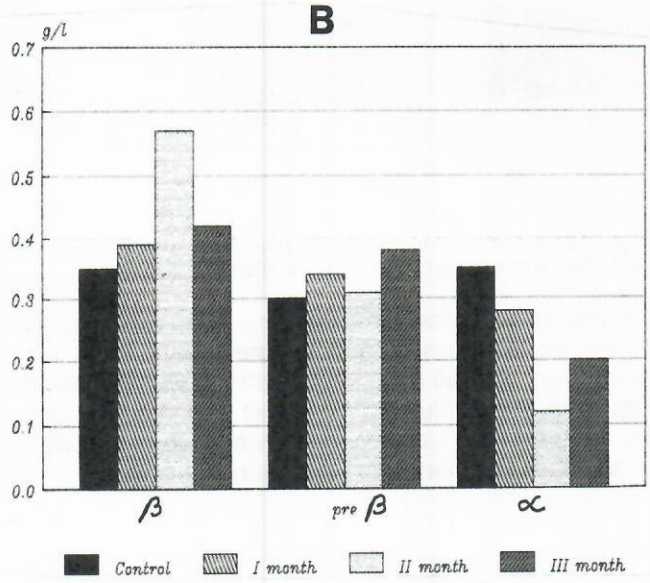


Figure 1. Serum lipoproteins (β, pre-β and α) after feeding different diets (g/l) (A) — high lipid diet



(B) — diet with sucrose

pulmonary arteries (Figure 3) (63 x HE). These changes are commonly associated with subendothelial edema with numerous vacuolae produced by ex-

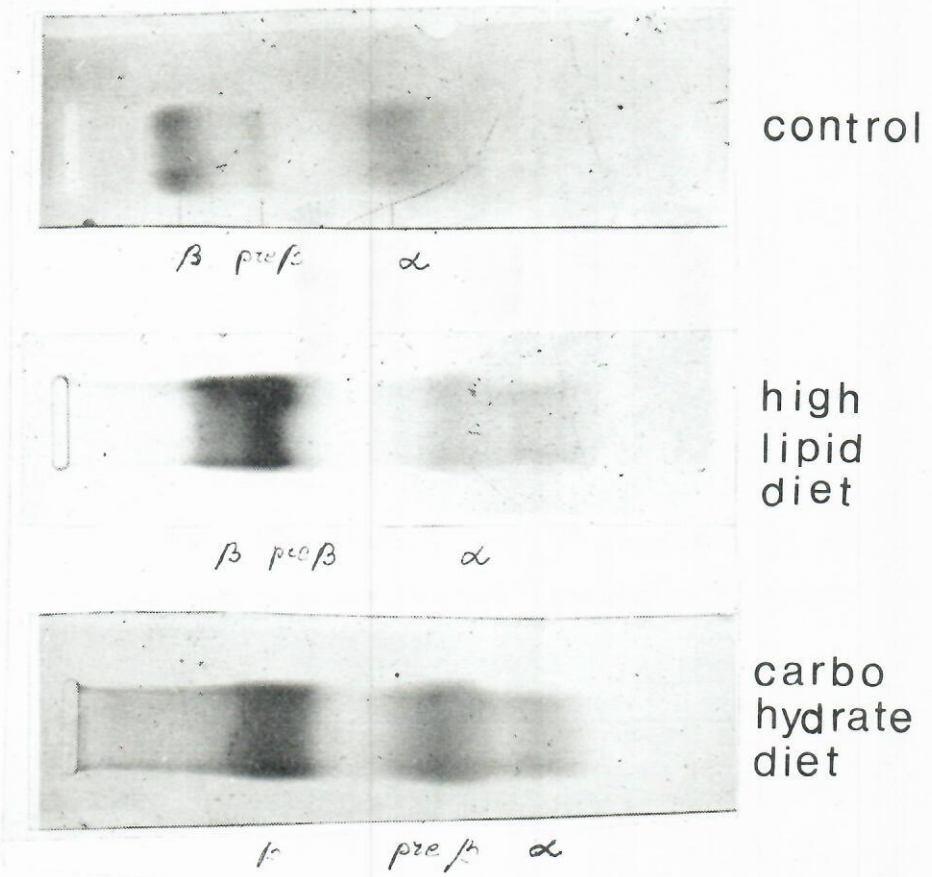


Figure 2. Electrophoregram of the lipoproteins (β, pre-β and α) in the serum after feeding different diets (control, high lipid diet and diet with sucrose).

traction of fat. Similar changes on the aorta have been reported in cholesterol-fed animals. Excellent visualization of accumulated fat in both intima and other layers of the aortic wall, as well as in vasa vasorum was achieved by the Sudan III method, (Figure 4) (200 x Sudan III). In this experimental group the earliest noted changes in the blood vessels were manifested as swelling of the endothelial cells and loss of boundaries between the cells.

DISCUSSION

Our studies have shown that aberrations from a normal diet in different animal species lead to significant changes in the metabolism of fats manifested as varous types of hyperlipidemia. Cholesterol and sucrose containing diets were examined in this study, and led to different types of hyperlipoproteinemia.

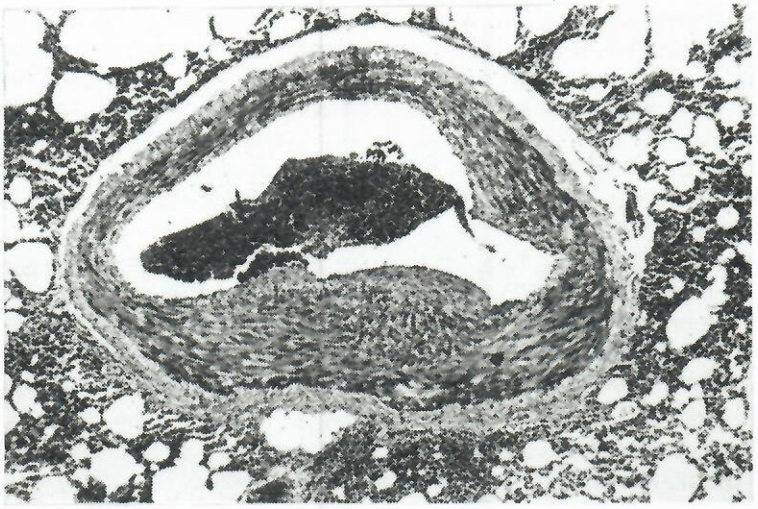


Figure 3. Fibrous thichening of the intima of the pulmonary artery (63xHE).

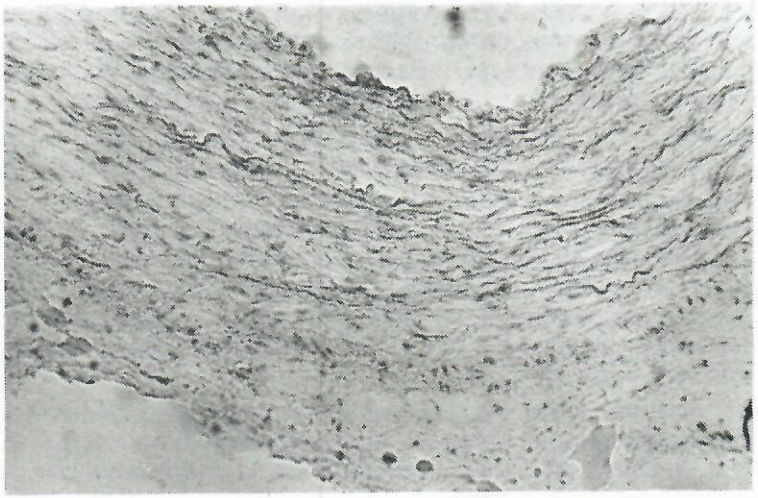


Figure 4. Visualization of accumulated fat in both intima and otherlayers, as well as in vasa vasorum of the aortal wall.

A load of saturated fats or cholesterol has a great influence on the enzyme systems regulating the metabolism of cholesterol in the liver, intestines and aorta. Both diets induced hypercholesterolemia. The effect of these two treatmens on the activity of hepatic and intestinal acyl-CoA: cholesterol acyltransferase and 3-hydroxy-3 methylglutary-CoA-reductase was measured. Ingestion of cholesterol stimulated esterification of cholesterol and inhibited synthesis of free cholesterol. Ingestion of saturated fats failed to affect the

synthesis of cholesterol, but it was noticed that it inhibited the esterification of cholesterol in both liver and intestines. A hypercholesterolemia-inducing diet is strictly related to an increase of the activity of acyl-CoA: cholesterol acyltransferase. Hypercholesterolemia induced by either of the diets tended to coat the aorta with cholesterol, and, therefore acyl-CoA: cholesterol acetyltransferase activity. It can be said that a change in esterification of cholesterol is directly related to the expected surge of cholesterol in all tissues (Jackson et al.,1990).

In the absence of activity of lecithin: cholesterol acyltransferase (LCAT) enzyme, free cholesterol (FC) may be transported from low density lipoproteins (LDL) and very low density lipoproteins (VLDL) to high density lipoproteins in the serum. This phenomenon can be important for excange of free cholesterol between serum lipoproteins and tissue cells (Velasquez et al., 1990).

Interestingly enough, medical therapy inhibits the develoment of atherosclerosis by selective blockade of the synthesis of apo-B proteins in the liver and intestines of experimental animals (Frolhkis et al., 1989).

Polynsaturated (n-3 and n-6) and saturated triglycerides influence the regulatory role of low density lipoproteins (LDL) and affect the dependant receptors and independent receptors of LDL transport. In an experiment with rats coconut oil was the basic food. When coconut oil was replaced with fish oil, lower levels of LDL were recorded than when sunflower oil was the substitute. This was attributed to the much higher increase of hepatic LDL-receptors than when sunflower oil was the substitute (Spady and Woollett, 1990).

Aplication of a carbohydrate supplemented diet in rabbits led to a rise of total serum cholesterol especially after the second month of this diet ($p<0.01$). The sucrose supplemented diet stimulated esterification of cholesterol, but not uniformly with all fatty acids. The intensity of esterification was much lower than in the cholesterol and oil supplemented diets. Nevertheless, the significant rise of total cholesterol in the first months of feeding with sucrose was noted to be reduced, after three months of the diets, to the level of significance for serum cholesterol values, suggesting production of large deposits of fat in tissues (Nedeljković and Šikić, 1992).

The cholesterol containing diet promoted an increase of serum cholesterol which led to the occurrence of hyperlipoproteinemia with an extremely elevated beta fraction which is a cholesterol carrier, ad well as a somewhat less prominent pre-beta fraction which is a carrier for endogenous triglycerides and occurred as the secondary effect of the cholesterol diet. The serum enriched with lipoproteins caused accumulattion of fats and penetration into the tissues of many organs which was demonstrated by histological methods.

Feeding rabbits with extra sucrose led to an increase of cholesterol already after the first month ($p<0.01$), and aspecially after the second month of this diet ($p<0.001$), which has already been reported in our previcus paper. Analysis of lipoproteins in the study showed promminent hyperlipoproteinemia. It can be stated that a carbohydrate diet promoted synthesis of cholesterol which was exibited in th lipoproteinogram of the serum as a very prominet beta

fraction. This condition induced by the carbohydrate diet was reflected in changess in the blood vessels of tissues and organs.

These diets, containing both cholesterol and carbohydrate, led to severe identical histological changes in the blood vessels of organs and tissues. This histological analysis showed atheromatous changes on the blood vessels of organs and tissues.

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EKSPERIMENTALNE HIPERLIPOPROTEINEMIJE KOD ZEČEVA IZAZVANE HOLESTEROLSKOM DIJETOM I DIJETOM UGLJENIM HIDRATIMA

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SADRŽAJ

Cilj rada bio je da se uporede hiperlipoproteinemije posle dijete saharozom i hiperlipoproteinemije koje nastaju posle holesterolske dijete.

Dijete koje su sprovedene na zečevima u istom vremenskom periodu u poređenju sa grupom zečeva na normalnoj ishrani pokazale su odstupanja od normalnog lipoproteinograma. Obe dijete su indukovale nastanak hiperlipoproteinemija koje su različitog tipa u zavisnosti od vrste dijete (ishrane).

Morfološke promene izazvane primenjenim dijetama bile su najizrazitije na krvnim sudovima. U holesterolskoj dijeti dominirale su promene u zidu aorte kao i u vasa vasorum, dok je dijeta saharozom izazvala oštećenja i u koronarnim krvnim sudovima, plućnim arterijama i arterijama nadbubrega.