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Invloeden op de hypercholesterolemie bij proefdieren en bij de mens

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1960

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Valkema, A. J. (1960). *Invloeden op de hypercholesterolemie bij proefdieren en bij de mens*. s.n.

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Download date: 08-06-2022

Summary

STUDIES ON THE DEVELOPMENT OF HYPERCHOLESTEROLEMIA IN ANIMALS AND IN MAN

Introduction

In this thesis dietary factors causing a reduction of an increased blood cholesterol content have been studied in animals and in man, with a view of finding substances that might be useful in the prevention of the atherosclerotic process.

Chapter I — The atherosclerosis problem

A short survey on the status of the problem of atherosclerosis is presented. From data available in the literature it can be supposed that in man a decrease of the serum cholesterol content to about 160 mg per 100 ml will be associated with a reduction in the occurrence of coronary atherosclerosis and its sequelae.

Chapter II — The cholesterol metabolism

The main factors determining the magnitude of the blood cholesterol pool: absorption, synthesis, catabolism, and excretion are especially surveyed.

Chapter III — Factors affecting the serum cholesterol level in man

The factors determining a normal serum cholesterol concentration and its deviations are reviewed. From existing data the conclusion is reached that blood cholesterol values higher than 160 mg per 100 ml are not necessarily a manifestation of the ageing process. Among the environmental factors influencing the serum cholesterol level the rôle of the dietary fat is pre-eminent.

Chapter IV — A possible cause of hypercholesterolemia in man

From a discussion on the blood cholesterol balance it becames probable that the net excretion of bile salts is an important factor controlling the serum cholesterol level in man. With a diet rich in saturated fats and poor in bulk the reabsorption of bile salts from the intestinal tract is increased and their excretion with the feces is decreased. The frequent recirculation of bile salts hinders the conversion of cholesterol to bile acids in the liver. This impairment in the catabolism of cholesterol may result in a hypercholesterolemia.

In the following three chapters our own investigations are described.

Chapter V — The effect of beta-sitosterol on blood cholesterol in the rabbit and in man

1. A pronounced increase of blood cholesterol readily develops in rabbits fed with a diet containing 0.5 per cent cholesterol. The effects of the addition of 2, 3 and 4 per cent beta-sitosterol to such a diet were studied. Sitosterol acted as a hypocholesterolemic agent (fig. 6). The addition of 4 per cent sitosterol completely prevented the increase in blood cholesterol (fig. 7).

2. The effect of beta-sitosterol as a possible means to achieve a reduction in elevated serum cholesterol levels in man was studied in detail.

In a preliminary experiment six patients received sitosterol in a dosage of 6 g daily. In all subjects a significant reduction in serum cholesterol was observed. Serum cholesterol rose on cessation of sterol feeding. In fig. 8 a typical serum cholesterol curve during sitosterol treatment is shown.

From control experiments, however, it appeared that the fluctuations in serum cholesterol levels, encountered in patients admitted to the hospital, were unexpectedly large. Therefore it was decided to re-study the action of sitosterol under carefully controlled clinical and dietary supervision in order to demonstrate whether or not the fall in serum cholesterol during the ingestion of sitosterol was greater than the 'spontaneous' variation in the serum cholesterol levels.

Nine patients received sitosterol after a control period. A dosage of 6 g daily did not produce a significant fall in the average serum cholesterol. In one of five patients the ingestion of 15 g of sitosterol per day resulted in a significant decrease of serum cholesterol.

The results of this study indicate that the feeding of beta-sitosterol is generally not effective in lowering serum cholesterol levels in man. There are conflicting reports in the literature concerning the effect of plant sterols as a means of lowering human serum cholesterol levels. These discrepancies may be due to the selection of patients with extreme fluctuations of serum cholesterol.

3. The literature on the effect of sitosterol on cholesterol absorption is reviewed. The interference with intestinal cholesterol absorption by sitosterol does not only concern the absorption of orally ingested cholesterol (500 mg per day) but also the reabsorption of biliary cholesterol (about 2000 mg per day). The fact, that sitosterol does decrease the absorption of cholesterol in man, but fails to have a significant effect on serum cholesterol levels, indicates that in man abnormal absorption as such is not the determining factor in the establishment of abnormal serum cholesterol concentrations.

4. It appears that the cholesterol-hypercholesterolemia in rabbits is not comparable with the hypercholesterolemia in man, and for this reason may not be the condition of choice in the search for a therapeutic hypocholesterolemic agent.

Rabbits also develop a hypercholesterolemia on cholesterol-free diets containing cholic acid or an excess of coconut fat. The effect of sitosterol on these hypercholesterolemias was also investigated. Six rabbits received a semisynthetic cholesterol-free diet containing 25 per cent coconut fat. After four weeks the mean serum cholesterol was increased to a constant level of 300 mg per 100 ml. The addition of 2 per cent sitosterol, dissolved in the coconut fat, produced a significant decrease in serum cholesterol (see fig. 9). Sitosterol, mixed as a powder through the semisynthetic diet of five rabbits, caused an equally rapid fall in serum cholesterol (fig. 10).

5. The serum cholesterol concentration in rabbits could be raised five times by adding 0.5 per cent cholic acid to a stock diet. The addition of 2 per cent sitosterol to this diet inhibited almost completely the hypercholesterolemia (fig. 11).

6. The mechanism of interaction of sitosterol in the coconut fat- and cholic acid-hypercholesterolemia is not clear from our experiments. An interference with the reabsorption of the cholesterol secreted with the bile into the gut is suggested. The resulting negative cholesterol balance apparently prevents the accumulation of cholesterol in the blood.

Chapter VI — The effect of some vanadyl compounds on blood cholesterol and atheromatosis in rabbits

1. Inhibition of endogenous cholesterol synthesis bears the promise of reducing the cholesterol content of the blood. A known inhibitor is the element vanadium. In 1956 Curran and Costello studied the effects of a vanadium compound in experimental atheromatosis. They were able to demonstrate the mobilization of predeposited cholesterol in the aorta's of rabbits. The cholesterol synthesis in the rabbit liver was strongly inhibited after administering nontoxic amounts of vanadyl sulfate (0.05 % $VOSO_4.2H_2O$) to the diet. When fed during 6 weeks immediately following a 4 week period of cholesterol feeding, the vanadyl sulfate diet reduced the high aortic cholesterol content by about 50 per cent.

In repeating this experiment we could completely confirm these results of Curran and Costello (table 10). The atheromatosis in the aorta decreased to the same extent as the excess aortic cholesterol. It was demonstrated that a cholesterol atheromatosis in rabbits does not regress spontaneously when the feeding of cholesterol is stopped. Two months after discontinuing the cholesterol feeding the aortic cholesterol content had actually become slightly higher.

2. We studied the effect of vanadium on the serum cholesterol and atheromatosis of the cholesterol fed rabbit. Vanadyl sulfate ($VOSO_4.5H_2O$) in a percentage of 0.05 % was added to the diet together with 0.25 per cent cholesterol. It was found (see table 11 and fig. 14) that serum, aorta, and liver levels of cholesterol were lower in the animals receiving vanadyl sulfate than in the control animals receiving 0.25 per cent cholesterol only.

3. With a view of administering vanadyl sulfate to human subjects we did some experiments in animals on the toxicity of vanadium

compounds. It soon became apparent, however, that vanadyl sulfate was not absorbed in man.

4. Four vanadyl compounds, which did become absorbed in man, were studied in rabbits. These substances were added in a percentage of 0.05 % to a rabbit ration containing 0.25 per cent cholesterol. After 100 days of feeding, the following mean serum cholesterol levels were found in groups of 6 to 10 rabbits receiving the compounds specified below:

no vanadyl compound	700 mg per 100 ml
vanadyl sulfate	400 mg per 100 ml
vanadyl tartrate	175 mg per 100 ml
diammonium vanadyl tartrate	700 mg per 100 ml
vanadyl citrate	170 mg per 100 ml
dicholine vanadyl citrate	500 mg per 100 ml

Diammonium vanadyl tartrate and dicholine vanadyl citrate had no effect on serum cholesterol and atheromatosis. With vanadyl tartrate low serum cholesterol values were observed and atheromatosis was practically absent (see table 12 and figure 15). The low serum cholesterol concentrations with vanadyl citrate, however, were not accompanied by a diminution in the occurrence of atheromatosis (see table 13 and figure 16). We cannot offer a satisfactory explanation for these conflicting results.

5. Vanadyl tartrate seemed to be the most promising compound for further research. The effect of the ingestion of this compound on the regression of predeposited aortic cholesterol was studied in rabbits. Vanadyl tartrate (0.08 per cent) fed during 124 days immediately following a 100 days period of 0.5 per cent cholesterol feeding, however, did not reduce excess serum and aortic cholesterol levels in a similar way as that observed with vanadyl sulfate (table 14).

6. Histologic examinations were carried out after every feeding experiment. Pathologic changes in liver, spleen, adrenal gland, heart and kidney have not been observed in any case.

7. The liver of rabbits receiving vanadyl sulfate contained in total about 10 γ of vanadium. When vanadyl tartrate was fed, values around 200 γ of vanadium were demonstrated. The liver vanadium concentration with dicholine vanadyl citrate was still higher. Doubling

the dose of a vanadyl compound (vanadyl citrate) caused a sixfold increase in the vanadium level of the liver.

8. These vagrant results in rabbits made us abstain from testing these vanadium compounds in humans.

Chapter VII — Experiments on the influence of bile acids on the blood cholesterol content

1. The nature of the dietary fat influences the concentration of the serum cholesterol. In man, the ingestion of corn oil causes a sharp fall in serum cholesterol, while rapeseed oil has only a moderately depressing effect. In rats, these fats act quite differently. A diet rich in rapeseed oil reduces the serum cholesterol to a lower level than one with corn oil.

In order to gain information about the mechanism of the above effect, we investigated the influence of the dietary calcium content. It is known that the absorption of rapeseed oil in rats is unfavourably affected by the relatively high calcium content of their diets.

Rats were fed a semisynthetic diet containing 25 per cent rapeseed oil. In repeated experiments it could be demonstrated that a reduction of the dietary calcium produced a significant elevation of serum cholesterol. With the normal calcium content, 1.5 per cent $CaCO_3$, the mean serum cholesterol values amounted to 66 mg per 100 ml and with 0.75 per cent $CaCO_3$ to 81 mg per 100 ml. The increased serum cholesterol levels were accompanied by a decrease in the proportion of fecal fat excreted as calcium soaps (table 16).

The inclusion of more than the normal quantity of calcium in the diet did not cause a further lowering of the serum cholesterol levels. The casein content of the semisynthetic diet appeared to be important. With 15 per cent casein the calcium effect was significant but not so with 20 or 10 per cent casein.

2. The low blood cholesterol values in rats on a rapeseed oil diet may be explained on the assumption that the presence of calcium soaps in the intestine hinders the reabsorption of bile salts.

To obtain proof for this assumption we investigated the effect of adding calcium soaps from rapeseed oil fatty acids to a diet rich in cholesterol in the rabbit. A significant hypocholesterolemic action could be demonstrated when the soaps were simultaneously fed with

antacids; the antacids prevented the decomposition of the calcium soaps in the acid content of the stomach. The low serum cholesterol levels were accompanied with a relatively high excretion of fat (calcium soaps) in the feces.

3. The addition of extra calcium to a human diet rich in rapeseed oil was investigated. In a preliminary experiment in 20 women the average serum cholesterol concentration sharply decreased with 20 mg per 100 ml after the administration of 1 g extra calcium (administered as $CaCO_3$ in a diet containing about 800 mg calcium). In a repeated experiment with the same subjects, however, no change in serum cholesterol was observed (fig. 20 and fig. 21). This apparent discrepancy remains as yet unexplained.

4. The feeding of 0.5 per cent cholic acid in a cholesterol-free diet to rabbits rapidly caused a hypercholesterolemia. Within two weeks the serum cholesterol regularly increased to a fairly constant level of about 200 mg per 100 ml (fig. 11).

This phenomenon can probably be ascribed to a reduction in the breakdown of cholesterol to bile acids.

5. The effect of the administration of cholic acid on blood cholesterol levels has also been investigated in man. The serum cholesterol did not change by the addition of 1.5 g cholic acid per day to an ordinary diet or to a diet in which 90 % of the mixed dietary fats had been replaced by corn oil.

However, the addition of cholic acid to a low-fat diet caused an increase of serum cholesterol. This experiment was carried out with 5 patients, whose usual diet contained approximately 100 g of mixed fats. The restriction of dietary fat to less than 25 g produced a decrease in serum cholesterol. With 1.5 g cholic acid daily their mean serum cholesterol level increased from 177 to 199 mg per 100 ml in the course of one week (fig. 23).

6. We believe that the results described in this chapter suggest that the metabolism, and especially the conditions governing the excretion and reabsorption of bile acids play an important role in the genesis of hypercholesterolemia in man.