Diet, Cholesterol Metabolism, and Atherosclerosis

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Abstract

Effects of diet on acetate incorporation into cholesterol and fatty acids in liver slices, and on the level of plasma cholesterol, were studied in rats and rabbits. Feeding fats and oils in a commercial diet stimulated acetate incorporation into rat-liver cholesterol much more than feeding them in a semisynthetic diet. This effect seemed to be specific for cholesterol since incorporation into fatty acids was not similarly affected. High levels of dietary casein inhibited acetate incorporation into both cholesterol and fatty acids. Rat-liver slices generally incorporated more acetate into cholesterol than rabbit-liver slices, but incorporation into fatty acids was often higher in the latter.

Rabbit plasma cholesterol were higher on butter diets than on corn oil diets. Further elevation of plasma cholesterol was observed when casein was added to the butter diet but not when it was added to the corn oil diet. Plasma cholesterol were elevated, and acetate incorporation into liver cholesterol and fatty acids was inhibited in suckling rats and rabbits whereas recently weaned animals gave results similar to those of adults. The inverse relationship between plasma cholesterol level and acetate incorporation into cholesterol may be attributable to feedback control of liver cholesterol biosynthesis. Other mechanisms which may account for the observed effects of dietary fats and protein on cholesterol metabolism, and the possible relevance of the findings to atherosclerosis, are discussed.

Introduction

Attempts to produce atherosclerosis in experimental animals were unsuccessful until the early years of this century when Ignatowski and others showed that rabbits develop atherosclerotic lesions when fed on meat, milk, and eggs (1-3). At first it was thought that the high protein content of these diets was responsible for the effect, but later experiments by Anitschlow and Chalatow (3,4) and by Wacker and Hueck (5) showed that similar results could be produced by feeding cholesterol. The focus of attention then centered on cholesterol, and much of the subsequent work on experimental atherosclerosis has been carried out with diets containing added cholesterol (3,6-10). Although this has yielded much valuable information, the amounts of cholesterol have, in general, been much greater than would be present in natural diets of either human beings or experimental animals (11), and such diets are therefore not satisfactory for exploring the metabolism of cholesterol under normal conditions. Further, it seems probable that the emphasis on dietary cholesterol has tended to obscure the effects of other dietary components which may play an important role in the etiology of atherosclerosis.

A significant development within the past 10 years has been the discovery that atherosclerotic lesions can be produced in rabbits by feeding diets containing little or no cholesterol (12-14). In general, synthetic diets appear to be more effective than stock diets in producing lesions under these conditions (15). The presence of fat in these diets is not essential, but the addition of saturated fat increases their potential for producing atherosclerosis (16). These results demonstrate that atherosclerosis can occur in the absence of dietary cholesterol, but the level of serum cholesterol nevertheless appears to be a significant factor in its development (17). Hypercholesterolemia usually precedes the formation of atherosclerotic lesions in experimental animals (6,8), and this is also true of rabbits fed low-cholesterol, semisynthetic diets (14). Since these rabbits have little access to exogenous cholesterol, it appears that their serum cholesterol must be derived almost entirely from endogenous synthesis. It is well known that many body tissues can synthesize cholesterol from small molecule precursors (18,19), but serum cholesterol appears to come mainly from the liver and intestine (20). In species such as the rat and dog, the liver seems to be the main source (21-23), but in human beings a larger proportion apparently comes from other tissues (24,25).

There is considerable evidence that liver cholesterol synthesis is controlled by a feedback mechanism regulated by cholesterol and bile acids (26-29). Synthesis can be inhibited by administering cholesterol or bile acids and may be increased by cannulating either the bile duct (30) or the intestinal lymph ducts (31), thus interrupting the enterohepatic flow of cholesterol and bile acids. There is lack of agreement on the extent to which this feedback mechanism operates in man (32,33), but, in experimental animals at least, it may be an important controlling factor in the regulation of serum cholesterol levels.

Effects of dietary components other than cholesterol on liver cholesterol synthesis have not been studied extensively, but the role of dietary fat has been investigated in a number of different laboratories (34-40). In most cases it has been found that dietary fat stimulates incorporation of acetate into liver cholesterol, and unsaturated fat appears to be somewhat more effective than saturated fat. Studies in this laboratory gave results in agreement with these conclusions, and it was found, in addition, that fats fed in a commercial diet gave much greater stimulation than fats fed in a semisynthetic diet (41). These effects of diet seemed to be specific for acetate incorporation into cholesterol since corresponding changes were not seen for acetate incorporation into fatty acids, measured in the same experiments (Figure 1).

Other studies in this laboratory showed that acetate incorporation into both cholesterol and fatty acids was reduced in liver slices from suckling rats (42). As soon as the animals were weaned, incorporation quickly increased to normal adult levels, and acetate incorporation into fatty acids by livers from recently weaned rats was frequently much higher than in adult livers although there was considerable variation from animal to animal (Figure 2). It seemed likely that the inhibition observed in suckling rat liver was related to diet because there was a rapid change at weaning, also because fetal rat liver was found to
RAT LIVER SLICE EXPERIMENTS.

ACETATE → CHOLESTEROL.

Dietary Fat. | Semisynthetic Diet. | Commercial Diet. |
---|---|---|
None. | | |
Coconut Oil. | | |
Butter. | | |
Olive Oil. | | |
Beef Tallow. | | |
Cottonseed Oil. | | |
Soybean Oil. | | |
Peanut Oil. | | |
Corn Oil. | | |
Sunflower Seed Oil. | | |

Per cent Incorporation.

Fig. 1. Effects of diet on incorporation of acetate-1-C\textsuperscript{14} into cholesterol and fatty acids by rat-liver slices. The basic composition of the fat-free semisynthetic diet is given in Table II. The commercial diet was a Fox Breeder Starter Ration, which was extracted with ether to remove most of its endogenous fat. All fats and oils were incorporated into the two diets at a level of 15\% by weight, and the diets were fed for approximately two weeks before the slice experiments were carried out. For further details see the original publication (41).

give good incorporation of acetate into cholesterol and fatty acids (42).

The in-vivo studies in which the labeled acetate was administered orally or intraperitoneally also showed that liver cholesterol and fatty acids accumulated less radioactivity in suckling rats than in weaned rats (42). Similarly, in vivo experiments with rats on semisynthetic and commercial diets (unpublished) confirmed these results of in vitro studies. The stimulating effect of dietary fat on acetate incorporation into liver cholesterol has also been observed with in vivo as well as in vitro experiments (34,40).

The experiments with suckling rats provided evidence of an inverse correlation between acetate incorporation into liver cholesterol and the level of plasma cholesterol since plasma cholesterol levels were elevated in suckling rats and dropped to normal adult values at weaning (Figure 3). There is some indication of a similar inverse correlation from experiments involving the feeding of semisynthetic and commercial diets. In the studies, acetate incorporation into cholesterol was lower in rats fed semisynthetic diets (Figure 1), but Portman and others have found that rats fed semisynthetic diets have higher serum cholesterol levels than rats fed commercial diets (43). Also, as mentioned earlier, rabbits develop hypercholesterolemia and atherosclerotic lesions more readily on semisynthetic diets than on stock diets. These findings and other evidence of a relationship between liver cholesterol metabolism, serum cholesterol levels, and development of atherosclerotic lesions (26,44) prompted further investigations of the influence of diet on acetate incorporation into liver cholesterol.

Earlier studies had shown that incorporation was markedly influenced by the presence of fat in the diet and the type of fat which was fed (Figure 1). However it seemed that the nonlipid portion of the diet must also have a significant role since the stimulation obtained by adding fats to a commercial diet was always much greater than that obtained by adding them to a semisynthetic diet.\textsuperscript{1} Therefore the main objective of subsequent experiments was the identification of nonlipid components of the diets which might

DECREASE IN RAT PLASMA CHOLESTEROL AT TIME OF WEANING.

In these experiments, most of the endogenous fat was first extracted from the commercial diet with ether, and experiments indicated that the small amount of lipid remaining was probably not responsible for the observed differences between commercial and semisynthetic diets.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Effects of diet on incorporation of acetate-1-C\textsuperscript{14} into cholesterol and fatty acids by rat-liver slices. The basic composition of the fat-free semisynthetic diet is given in Table II. The commercial diet was a Fox Breeder Starter Ration, which was extracted with ether to remove most of its endogenous fat. All fats and oils were incorporated into the two diets at a level of 15\% by weight, and the diets were fed for approximately two weeks before the slice experiments were carried out. For further details see the original publication (41).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Incorporation of acetate-1-C\textsuperscript{14} into cholesterol and fatty acids by liver slices from suckling and weaned rats. The animals were 13 to 17 and 30 to 35 days old respectively. There were six animals per group.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Free and total plasma cholesterol levels in suckling and weaned rats. The animals were weaned at 20 to 21 days of age.}
\end{figure}

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