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EFFECT OF LYMPHATIC DRAINAGE ON 7α-HYDROXYLATION OF CHOLESTEROL IN RAT LIVER

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SUMMARY

It was recently shown that dietary triglycerides of different degree of unsaturation had different effects on 7α-hydroxylation of cholesterol in rats (Björkhem, I., Blomstrand, R., Svensson, L., J. Lipid Res. 19, 359 (1978)). It is shown here that lymphatic drainage in general stimulates 7α-hydroxylation of cholesterol about two-fold regardless of presence or absence of triglycerides in the diet or type of triglycerides. Thus the major differences between the effect of different triglycerides are retained after lymphatic drainage. These results, together with previous results, support the contention that at least part of the effects of different dietary triglycerides on 7α-hydroxylation of cholesterol is related to differences in absorption from the intestine. The possibility is discussed that chylomicrons from the lymph have a direct inhibitory effect on 7α-hydroxylation of cholesterol.

According to current concepts, the rate-limiting step in bile acid biosynthesis, 7α-hydroxylation of cholesterol, is regulated primarily by the amounts of bile acids returning to the liver (1). The most obvious evidence is the fact that biliary drainage leads to a severalfold increase in enzyme activity. Since a biliary drainage also leads to a decreased absorption of fat, it is possible that not only bile acids but also absorbed fat may have some inhibitory effect on 7α-hydroxylation of cholesterol. It has been reported that biliary obstruction for 48 hours in rats leads to an increase in cholesterol 7α-hydroxylase activity (2, 3). A possible explanation for this is a decreased inhibition by chylomicrons in the lymph.

In order to study a possible effect of absorbed fat on 7α-hydroxylation of cholesterol, we recently assayed 7α-hydroxylase activity in rats fed a diet containing 20% triglycerides

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of different degree of unsaturation (4). It was found that the 7α-hydroxylase activity was two- to four-fold higher in rats fed tripalmitin diet than in those fed trilinolein diet. Very recently, similar effects of saturated and unsaturated triglycerides were observed also on hepatic HMG-CoA reductase activity in rats (5). The differences obtained may either be due to differences in direct effect on hepatic enzyme or differences in degree of absorption from the intestine. In the latter case a diminished absorption of fat may lead to an increased loss of bile acids in faeces with a subsequent decreased feed-back inhibition of cholesterol 7α-hydroxylase and possibly also hepatic CoA-reductase.

In an attempt to discriminate between the two possibilities, we have now studied the effect of lymphatic drainage on 7α-hydroxylation of cholesterol in rats fed fat-free diet and diets with different composition of fat. If the different effects of the triglyceride-containing diets on 7α-hydroxylation of cholesterol are due to direct effects on the hepatic enzyme, lymphatic drainage can be expected to eliminate the differences. If the different effects are due to differences in the degree of absorption, however, the differences in effect on 7α-hydroxylation of cholesterol should be retained also after lymphatic drainage.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 250 g were used in the study (Anticimex, Stockholm, Sweden). In order to assay 7α-hydroxylase activity under optimal conditions (6, 7) all animals were subjected to reversed lighting period (light was automatically switched on at 6 p.m. and off at 6 a.m.) The animals were fed the semisynthetic diets for four weeks (cf. ref. 4) which differed only in concentration or type of fat. The composition of the diets were as follow: fat 0 % or 20 %, corn starch 50 % or 30 %, casein 26 %, cellulose powder 4 %, dextrose 10 %, $CaCO_3$ 2 %, NaH_2PO_4 3 %, salt mixture (mixture number 7, method 400, Astra Ewos AB, Södertälje, Sweden) 4 %, vitamin mixture (complete, for rat and mouse, Astra Ewos AB) 1 %. The type of fat used was either safflower oil (containing 77 % linoleic acid) or tripalmitin (purity 95 %). For details concerning composition of fat and gain of weight of rats on these diets -

see ref. 4. One group of rats were fed a commercial pellet diet containing 4.5 % fat (standard food for rat and mice, Astra Ewos AB). A lipid extract from each semisynthetic diet contained less than 0.2 % sterols (4). After four weeks on the diet, the animals were operated under ether anaesthesia and the thoracic lymph duct cannulated just proximal to the cisterna magnum through an abdominal approach (8, 9). The proximal part of the cannulated duct was ligated. The animals were then kept in restraining cages for 50-60 hours while the lymph was drained. All animals were sacrified at the same time in the morning (8-9 a.m.), blood samples were drawn and the liver removed. The control animals were treated in the same way as above but only sham-operated with a similar incision as above through the abdominal wall. In the cages the rats with a lymphatic fistula were fed the same diet as above but instead of water given 0.6 % (w/v) NaCl to drink freely. No animals surviving operation died or showed signs of complication during the period of lymph sampling. The sham-operated animals were given 0.3 % (w/v) NaCl to drink freely.

Preparation of liver homogenate incubation procedure and mass fragmentographic determination of 7α-hydroxycholesterol was performed as described previously (4). Protein was determined according to the procedure of Lowry et al.(10). Triglycerides and cholesterol were determined according to enzymatic methods (11, 12).

RESULTS

Table 1 summarizes the results. In accordance with the previous study (4), control animals on the trilinolein diet had considerably lower 7α-hydroxylase activity than control animals fed the fat-free diet, the tripalmitin diet or the commercial pellet diet. The lymph fistula was found to stimulate the activity about two-fold in the rats fed the trilinolein diet, the fat-free diet and the commercial pellet diet. This stimulation was statistically significant ($p < 0.02$, Student's t-test). In rats fed the tripalmitin diet, there was only a slight and statistically insignificant stimulation of 7α-hydroxylation of cholesterol by the lymph fistula ($p > 0.05$). The individual variations were, however, much greater in the experiments with rats fed the tripalmitin diet than in the experiments with rats fed the other diets.

Table I

Effect of lymphatic drainage on 7α-hydroxylation of cholesterol †

Animals	Cholesterol 7α-hydroxylase activity nmol/mg protein / 15 min	
Tripalmitin diet		
controls	0.492 $\pm$ 0.077	
lymph fistulated	0.579 $\pm$ 0.074	$p>0.05$
Trilinolein diet		
controls	0.122 $\pm$ 0.021	
lymph fistulated	0.292 $\pm$ 0.089	$p<0.005$
Fat-free diet		
controls	0.198 $\pm$ 0.016	
lymph fistulated	0.405 $\pm$ 0.026	$p<0.0006$
Commercial pellet diet		
controls	0.223 $\pm$ 0.021	
lymph fistulated	0.446 $\pm$ 0.076	$p<0.02$

† Mean $\pm$ S.E.M. of results from six animals

The cholesterol 7α-hydroxylase activity given in Table I was assayed by the mass fragmentographic method which gives conversion of endogenous cholesterol into 7α- hydroxycholesterol. The same effects were, however, obtained when the activity was assayed by following conversion of exogenous 4-^{14}C-cholesterol into 4-^{14}C-7α-hydroxycholesterol (results not shown in Table).

The lymph fistula was found to decrease the concentration of albumin in serum in all cases. This decrease was statistically significant ($p <0.005$). Serum cholesterol concentration was not affected to a significant degree. Serum triglycerides were not affected by the lymph fistula in the rats fed tripalmitin or fat-free diet. In the rats fed the trilinolein diet, however, the lymph fistula decreased the concentration of serum triglycerides significantly ($p <0.0005$) (Table 2).

Table 2

Serum concentration of triglycerides, cholesterol and albumin in the different groups of rats †

Animals	S-cholesterol mmol/l	S-triglycerides mmol/l	S-albumin g/l
Tripalmitin diet			
controls	2.8 ± 0.2	0.65 ± 0.24	33 ± 2
lymph fistulated	2.8 ± 0.2	0.48 ± 0.16	26 ± 1
Trilinolein diet			
controls	3.7 ± 0.1	1.12 ± 0.06	37 ± 1
lymph fistulated	3.8 ± 0.1	0.58 ± 0.04	30 ± 1
Fat free diet			
controls	2.2 ± 0.2	0.42 ± 0.09	32 ± 1
lymph fistulated	2.6 ± 0.1	0.43 ± 0.04	28 ± 1

† Mean ± S.E.M. of results from six animals

As shown in Table 3, the lymphatic flow was highest in the rats treated with tripalmitin and lowest in the rats fed the fat-free diet. Loss of cholesterol in the lymph was highest in the rats fed trilinolein diet. Loss of triglycerides was also highest in the rats fed the trilinolein diet. Loss of triglycerides in the lymph of rats fed the tripalmitin diet could not be determined accurately with the method used due to flocculation.

DISCUSSION

It is evident from the present results that the major differences between the different types of diets with respect to effect on 7α-hydroxylation of cholesterol were retained after lymphatic drainage. It may therefore be concluded that at least part of the effect of the different dietary triglycerides on 7α-hydroxylation of cholesterol observed in the previous work (4) are due to differences in the absorption. This contention

Table 3

Lymph flow and loss of cholesterol and triglycerides through the lymph fistula †

Animals	Lymphatic flow ml/24 h	Loss of cholesterol µmol/24 h	Loss of triglycerides mmol/24 h
Tripalmitin diet	160 ± 20	48 ± 9	<1[α]
Trilinolein diet	107 ± 8	80 ± 10	3.74 ± 0.5
Fat-free diet	70 ± 13	44 ± 8	0.16 ± 0.0

† Mean ± S.E.M. of results from six animals

α Due to flocculation no accurate determination of triglycerides in lymph could be performed in this case

is supported by the fact that the highest stimulation of 7α-hydroxylation in the previous work was found with diets containing fatty acids which are absorbed less readily from the intestine in rats (palmitic acid and erucic acid). The lowest level of 7α-hydroxylation was found with fatty acids which are very efficiently absorbed from the intestine, such as linoleic acid and lauric acid. It may be mentioned in this connection that the volume of faeces was much greater from the rats treated with the tripalmitin and the trierucin diets than from those treated with the trilinolein diet. It thus seems probable that loss of fatty acids with faeces is coupled with a higher loss of bile acids than normal. A higher loss of bile acids with faeces leads to a decreased inhibition of the cholesterol 7α-hydroxylase, and possibly also the HMG-CoA reductase.

The possibility can not be completely excluded that the feeding with some of the diets may cause irreversible changes in the enzyme system under study. This may be the explanation for the failure to stimulate 7α-hydroxylation to a significant degree with a lymph fistula in the rats treated with tripalmitin.

Further experiments designed to study this possibility are in progress.

In the evaluation of the present results it must be borne in mind that relatively drastical experimental conditions were used. Thus a diet containing 20 % tripalmitin may be regarded as unphysiological. In previous work (8, 9), it has been shown that a small part (3-5%) of ^{14}C-labeled long-chain fatty acids fed to rats with a lymphatic fistula are not recovered in the lymph. In spite of the experimental limitations, however, the fact that lymphatic drainage stimulated 7α-hydroxylation of cholesterol under most dietary conditions, may be of interest from a regulatory point of view.

The degree of stimulation was not related to the volume of the lymph flow, the magnitude of loss of triglycerides or cholesterol through the lymph, or the nutritional status as judged from serum cholesterol, serum triglycerides and serum albumin. One possibility is that the lymph flow from the fistula by some unknown mechanism per se is a stimulus for 7α-hydroxylation and that this stimulus is unrelated to the lipids in the lymph. The fact that a stimulation was observed also in rats fed a fat-free diet can not be taken as evidence for such a hypothesis, since the lymph always contains endogenous lipids also when no fat is fed. It seems more probable that the stimulatory effect of lymphatic drainage on 7α-hydroxylation of cholesterol is due to a released inhibition by chylomicrons on the enzyme system. Such a hypothesis is in good agreement with the previous finding that biliary obstruction leads to a stimulation of 7α-hydroxylation of cholesterol (2, 15). If this hypothesis is valid, the inhibition by chylomicrons seems to be of "all or nothing" type. Thus the degree of inhibition should be about the same regardless of whether small or great amounts of chylomicrons reaches the liver.

According to Weis and Dietchy, also biosynthesis of cholesterol is inhibited by chylomicrons reaching the liver (13). Thus lymphatic drainage was found to increase hepatic biosynthesis of cholesterol from acetate 2.5- to 3-fold. A similar increase was also found after biliary obstruction. It should be mentioned, however, that Hamprecht and collaborators did not find a stimulatory effect of lymphatic drainage on hepatic MHG-CoA reductase activity in rats (14). There seems to be a tight coupling between biosynthesis of cholesterol and 7α-hydroxylation of cholesterol under several different conditions (1, 15). In some studies it has been reported that an increase in 7α-hydroxylase activity is preceeded by an increase in HMG-CoA activity (1, 16). It can thus not be excluded that the stimulatory effect of lymphatic drainage on 7α-hydroxylation observed in the present work is secondary to an increased biosynthesis of cholesterol similar to that observed by Weis and Dietchy. At the present state of knowledge, however, such a relationship between the two activities is difficult to understand. It has been suggested that the postulated inhibitory effect of chylomicrons on biosynthesis of cholesterol is due to their content of cholesterol (13), and it is well known that cholesterol feeding inhibits HMG-CoA reductase activity. Cholesterol feeding does not inhibit hepatic 7α-hydroxylase activity, however, (13, 15). In some studies even a stimulatory effect has been observed (17-19).

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