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Substrate Incorporation into Lipids and Proteins in Human Liver Slices

An experimental study with special reference to dyslipoproteinemic conditions

By Håkan Stakeberg

Göteborg 1974

This paper is also published as supplement no. 561 to Acta Medica Scandinavica.

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AKADEMISK AVHANDLING

SOM FÖR VINNANDE AV MEDICINE DOKTORSGRAD,
MED VEDERBÖRLIGT TILLSTÅND AV MEDICINSKA FAKULTETEN
VID UNIVERSITETET I GÖTEBORG, KOMMER ATT OFFENTLIGEN
FÖRSVARAS I FÖRELÄSNINGSSAL F 3, SAHLGRENSKA SJUKHUSET,
FREDAGEN DEN 3 MAJ 1974 KL. 9.00 F.M.

Av

HÅKAN STAKEBERG
MED. LIC.

GÖTEBORG 1974

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Substrate Incorporation into Lipids and Proteins in Human Liver Slices

An experimental study with special reference to dyslipoproteinemic conditions

By

HÅKAN STAKEBERG

GÖTEBORG 1974

Tryckt i offset
Elanders Boktryckeri Aktiebolag Kungsbacka 1974

To Kerstin

Johan, Johanna o. Josefina

This thesis is based on the following papers:

I Stakeberg, H., Gustafson, A. & Scherstén, T.: Incorporation rate of leucine into proteins in human liver slices. Submitted for publication in Europ. J. Clin. Invest.

II Stakeberg, H., Lundborg, H. & Scherstén, T.: Incorporation rate in vitro of precursors into hepatic lipids and proteins in patients with extrahepatic cholestasis. Submitted for publication in Europ. J. Clin. Invest.

III Stakeberg, H. & Scherstén, T.: Substrate incorporation into hepatic lipids and proteins in vitro in patients with prebeta hyperlipoproteinemia. Submitted for publication in Scand. J. Clin. Lab. Invest.

IV Cahlin, E., Jönsson, Jane, Persson, B., Stakeberg, H., Björntorp, P., Gustafson, A. & Scherstén, T.: Sucrose feeding in man. Effects on substrate incorporation into hepatic triglycerides and phosphoglycerides in vitro and on removal of intravenous fat in patients with hyperlipoproteinemia. Scand. J. Clin. Lab. Invest. 32, 21, 1973.

These papers will be referred to by their Roman numbers.

CONTENTS

INTRODUCTION

In 1950, J.W. Gofman (35) suggested that coronary heart disease (CHD) was associated with high plasma levels of certain lipoprotein density classes. Since then, retrospective (17, 18, 22, 27, 40) as well as prospective studies (12) have disclosed that several types of hyperlipoproteinemia are risk factors in the development of CHD (for reviews, cf. 93, 13).

A great deal of work has been done to classify these hyperlipoproteinemic conditions and to elucidate their etiological mechanisms (for reviews, cf. 32, 56).

Generally speaking, an increased concentration of circulating lipoproteins may be the result of an increased production rate, a decreased removal rate, or a combination of both. The liver is the primary site of the synthesis and secretion of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) (46). The removal of VLDL, on the other hand, is at least partly dependent on the activity of adipose tissue lipoprotein lipase (75). It is now accepted that, in patients with endogenous hyperlipoproteinemia, the VLDL concentration (often referred to as prebeta lipoproteins) in the plasma may be increased and maintained after sucrose feeding (for reviews, cf. IV). Most information on the effect of dietary carbohydrates on the lipid metabolism, however, is derived from studies in animals. Furthermore, the kinetic behavior of plasma and liver lipids has, in many instances, indicated marked species differences (2, 28, 39, 57) and many results obtained from animal studies cannot be extrapolated directly to man.

Most investigations in human beings on the mechanisms of dyslipoproteinemic conditions have applied a kinetic approach. Thus, either isotope-labeled glycerol or fatty acids have been administered intravenously as precursors for the endogenous hepatic lipoprotein synthesis and secretion into the blood, after which the decay of lipoprotein radioactivity has been followed. The results of these studies have been conflicting, however, partly because of methodological problems. This has recently been critically reviewed by Nikkilä et al. (55, 56).

So far, a great deal of knowledge has been gained about the production and removal of plasma lipids. On the other hand, only sparse information with respect to the metabolism of the protein part of the lipoprotein is available. It has been suggested that the synthesis rate of carrier proteins (apoproteins) may be of importance for at least the VLDL secretion rate from the liver (14, 32, 46, 62).

This study was designed to obtain more direct information regarding the contributive role of the liver in the genesis of different dyslipoproteine-

mic conditions in man.

Extrahepatic cholestasis in man is associated with well-defined changes in the plasma lipoprotein pattern (33, 69, 75, 76, 77, 83, 90). These changes are suggested to be at least partly secondary to a changed hepatic lipid- and protein metabolism as indicated by studies in animals (47, 73, 92) and in man (86). Therefore, patients with this disorder were studied and served as models for a dyslipoproteinemic condition with a liver metabolic involvement. The main purpose of this investigation, however, was to characterize and evaluate the role of the hepatic protein and lipid synthesis rate in the production of increased plasma concentrations of VLDL in patients with prebeta hyperlipoproteinemia.

An in vitro technique with human liver slices to determine the triglyceride and phosphoglyceride synthesis capacity has previously been described by Nilsson & Scherstén (58) from this laboratory. Another aim of this study, therefore, was to work out and characterize a method to measure the capacity of human liver slices to incorporate amino acids into proteins as an expression of the protein synthesis rate.

METHODOLOGICAL CONSIDERATIONS

Anesthesia and liver biopsy

The patients were premedicated one hour before the introduction of general anesthesia by means of diazepam and atropine. Anesthesia was given as hexobarbital (Evipan^{R}), nitrous oxide, oxygen and succinyl choline. Liver tissue for in vitro studies was obtained by a biopsy technique according to Björntorp et al. (9). The technique was described in detail by Gottfries (36). The biopsies were performed immediately after the abdominal cavity was opened and the excised liver tissue was placed in ice-cold buffer solution and rapidly brought to the laboratory ($\leq$ 5 min.).

Determination of the incorporation rate of leucine into proteins in human liver slices (I, II, III).

Different preparations to determine the amino acid incorporation rate in vitro into hepatic proteins in experimental animals, e.g. subcellular systems (44, 61), suspensions of free cells (82), liver slices (20, 21, 68) and perfused liver (45, 53) have been described. As a very complicated sequence of reactions, such as the protein synthesis is studied, it is desirable to preserve the integrity of the liver tissue as much as possible. For obvious reasons, the organ preparation representing the highest degree of integrity, the perfused liver, could not be chosen for studies in man. Moreover, the tissue slice technique gives, if not quantitatively, so at least semiquantitatively, the same information, provided that the basic conditions with respect to the influence of the substrate composition and concentration are well characterized (68).

Using the technique described in detail in Paper I, it was possible to determine, with sufficient accuracy, the incorporation rate of leucine into soluble proteins in human liver tissue slices. It was demonstrated that zero order kinetics were obtained for at least four hours when 500 mg liver slices were incubated in a "complete amino acid" medium. The incorporation rate of leucine into proteins revealed saturation kinetics at a concentration four times that of the serum of a complete amino acid mixture in the incubation medium. In this system, we found an intracellular leucine concentration of the same magnitude as in the medium (based on calculations of determined inulin space and diffusible water). According to Peters et al. (68) and Mortimore et al. (53), as reviewed in Paper I, the specific activity of the precursor amino acid in the medium may be valid for the calculation of the protein synthesis rate under these conditions.

Addition of insulin to the medium (100 mU/ml) increased the incorporation rate of leucine by 32 % in three consecutive experiments, whereas

supplementation with glucose (5.5 mmol/l) or fructose (5.5 mmol/l) caused no change, or an inhibition, of the incorporation rate of leucine, respectively. These findings are in good agreement with previous studies in experimental animals (11, 51, 54) and indicate that the technique described is adequate to reveal changes in the incorporation rate of leucine into liver proteins in man at least as accurately as previously described for experimental animal liver tissue preparations.

Leucine is metabolized in the liver to a low extent. Thus, label was found in CO_2, aceto-acetate, and in lipids. This incorporation into various metabolites represented a small fraction, however, which agrees with other reports (26, 68). This finding probably reflect the relative inability of the liver to degrade branched-chained amino acids, which in turn makes them suitable as labeled precursors in in vitro studies of the present kind.

The standard error of the method for the incorporation rate of leucine into proteins, calculated on the basis of 38 duplicates, was ± 9.5 per cent, and for the isolation of proteins from liver tissue ± 4 per cent.

Determination of the incorporation rates of glycerol and fructose into glycerides, and into glycogen in human liver slices (II, III, IV).

The technique used for this purpose was worked out in this laboratory several years ago (58). For details concerning the incubation procedure see Paper IV.

The basic incubation medium consisted of 3 per cent albumin in Krebs Ringer phosphate buffer, pH 7.4. Palmitic acid, glycerol, and fructose were added to this medium to obtain the appropriate concentration of these substances in each individual experiment. As radioactive precursors for the lipid synthesis, glycerol-1,3-^{14}C or fructose-U-^{14}C were used. The incubation time was four hours. The incorporation time curve for glycerol and fructose into phospholipids and triglycerides was linear for at least five hours (58). After incubation, the liver slices were submerged to 10 ml chloroform-methanol (2/1, v/v). After 22 hours, the extract was washed with 10 ml of 0.05 M KCl by a modification of the method of Folch et al. (30).

The standard error for the incorporation rate of glycerol into lipids was determined on the basis of a series of duplicates and was found to be ± 9 per cent.

The lipid synthesis was calculated from the specific activity of the precursor in the medium and recovered radioactivity in the glycerides and expressed as nmol incorporated glycerol or fructose per gram liver per hour.

Analytical procedures

Separation of different lipids in the chloroform-methanol extracts from plasma and liver tissue was performed by thin-layer chromatography (TLC)

according to Gloster & Fletcher (34). The determination of lipid phosphorus was performed according to the Bartlett method (5) as modified by Svennerholm & Vanier (89) (standard error of the method 5.0 per cent). Glyceride-glycerol was determined as described by Carlson (19) (error 3.3 per cent), cholesterol according to Sperry & Webb (85) (error 10.4 per cent), glucose according to Levin et al. (50) and glycogen according to van der Vies (94) (error 4.2 per cent). IRI-insulin was determined radio-immunochemically according to Hales & Randle (41) using assay kits from Amersham (insulin immuno assay kit, the Radiochemical Center, Amersham, Buckingham, England) (error 7.1 per cent). Protein was quantitated according to Lowry et al. (49) (error 2 per cent).

The radioactivity was determined in a Packard Tri-Carb (3320) liquid scintillation spectrometer. Correction for quenching was performed by the external standard method.

Experimental procedures in patients on a carbohydrate-enriched diet (III, IV).

All patients on a carbohydrate-enriched diet were hospitalized for 20 days and the patient group designated as non-sucrose-fed controls for two days prior to the gallbladder operation. In the morning on the day after admission, after an overnight fast, capillary blood and heparinized venous blood samples were drawn for subsequent determination of blood glucose (50), basal plasma insulin (41), plasma cholesterol (85), and triglycerides (19), and for lipoprotein electrophoresis (71). A glucose tolerance test was performed the same day as described in detail in Paper IV.

In the morning of the second day, the intravenous fat (Intralipid^{R}) tolerance test (10) was performed with the patient in the fasting state. Just before the tolerance test, a percutaneous biopsy of adipose tissue was taken from the gluteal region (66). The samples obtained were used to determine the lipoprotein lipase activity (67).

The patients were given 200 g sucrose per day from the third day through the 17:th day. The ordinary hospital diet contained an average of 2500 Kcal, distributed as 500 Kcal of protein, 900 Kcal of fat, and 1100 Kcal of carbohydrates, mainly as starch. With the aid of a dietician, approximately 800 Kcal (160 Kcal protein, 290 Kcal fat, and 350 Kcal carbohydrates) of the daily food intake was replaced by 200 g of sucrose. The sucrose solution, kindly supplied by the Beacham Company, London, England, was flavored and dispensed in 100 g bottles.

Throughout the sucrose feeding period, blood samples were taken every second day to determine plasma cholesterol and triglycerides. After the sucrose feeding period, but before the gallbladder operation, a second glucose tolerance test was performed on the 17:th day. The fat tolerance test, with a concomitant determination of the adipose tissue lipoprotein lipase activity,

were repeated on the 18:th day. These determinations were performed under the same conditions as before the sucrose feeding period.

Statistical methods (I, II, III, IV)

In the comparison between different groups, Student's t-test was used. For pair differences, Wilcoxon's test as described by Siegel (84) was used. Linear regressions were calculated according to the method of least squares. Standard procedures were also used to calculate correlation coefficients. T-tests were applied to check the significance of regression coefficients. Standard error of the methods was calculated from duplicates using the following formulae:

$$SE = \sqrt{\frac{\sum d^2_i}{2\,n}}$$

where d_i is the difference between duplicate measurements. The error was then expressed as a percentage of the mean of all determinations.

CLINICAL MATERIAL

The clinical material consisted of 105 subjects, 38 men and 67 women aged 20-86 years. All patients except those with extrahepatic cholestasis (II) had a normal liver function according to the following criteria: serum bilirubin $\leq$ 1.0 mg/100 ml; thymol turbidity $\leq$ 0.10, alkaline phosphatase (EC 3.1.3.1.), $\leq$ 10 units (Buch); L:aspartate:2-oxoglutarate (EC 2.6.1.1.) $\leq$ 20 units ($NAD/NADH_2$).

None of the patients had any known disease apart from the gallbladder disease and, in pertinent cases, prebeta hyperlipoproteinemia (except Patient No. 1, Paper III, who had slight maturity onset diabetes mellitus treated with sulfonylurea). At the time of the study or shortly before, none was taking any drug known to affect the liver function. Patients with known or suspected alcoholic problems and women taking oral contraceptive steroids were excluded from the study.

The patients were divided into different groups on the basis of their initial plasma lipoprotein pattern according to Fredrickson & Lees (6, 31). The plasma lipoprotein type was determined from repeated plasma lipid analyses, lipoprotein electrophoresis on agarose gel (71), and alphalipoprotein cholesterol determinations (6). Based on these analyses, determinations of prebeta lipoprotein and beta lipoprotein cholesterol were performed according to Gustafson et al. (40). The lipoprotein values were used for the subsequent lipoprotein typing. The presence, at repeated analyses, of prebeta lipoproteins at agarose gel electrophoresis, concomitant with $>$ 35 mg/100 ml of prebeta lipoprotein cholesterol, were used as definition of prebeta hyperlipoproteinemia. The corresponding upper limit for beta lipoprotein cholesterol was set at 200-220 mg/100 ml, dependent on the patient´s age and sex.

The group with prebeta hyperlipoproteinemia (n = 21) comprised 6 patients with hyperlipoproteinemia, Type II B, and 15 patients with Type IV. All controls were admitted to the hospital for an operation for uncomplicated gallbladder disease. The plasma triglyceride and cholesterol concentrations in the control group were not different from those of middle-aged men and women randomly selected from the same city (8).

Fifteen of the patients studied had extrahepatic cholestasis (II). Clinical data on the time of the liver biopsy and the blood sampling are reported in detail in Paper II. In all patients, the diagnosis was confirmed at laparotomy and in the five tumor patients by means of microscopy. No detectable metastasis in lymph nodes or liver was found in any of these patients. Patients Nos. 1, 3, and 11 had localized carcinoma of the head of the pancreas, strangulating the common bile duct, while patients Nos. 2, and 14 had small,

isolated bile duct carcinomas.

Consent was obtained from all patients on the carbohydrate-enriched dietary regimen. No complications in connection with the liver biopsies or the other procedures were encountered.

Incorporation rate of leucine into hepatic proteins in vitro in normolipoproteinemic controls (I).

The incorporation rate of leucine into proteins in liver slices from 42 normolipidemic gallstone patients was studied with the technique described in detail in Paper I. No sex differences were found. In subjects older than 60 years, we found a higher ($P < 0.025$) incorporation rate of leucine into proteins than in subjects below the age of 60. Since the protein content in the liver tissue from these subjects was approximately the same as in the younger ones, this finding may be interpreted as a more rapid hepatic protein turnover rate in older subjects. This would be in line with the high lysosomal enzyme activity in aging human liver tissue (7) and is in agreement with the finding of an increased synthesis rate of albumin in vivo in the old rat (64).

Provided the incorporation rate of leucine into liver proteins can be taken as an expression of protein synthesis, it is possible to calculate the synthesis rate by dividing the amount of leucine incorporated into hepatic proteins by the mass fraction of leucine in liver proteins 0.09 (w/w) (52). This will give a synthesis rate of 0.100 mg protein per hour per gram liver tissue, which is roughly 1/10 of the corresponding synthesis rate for rat liver in vitro (68). Our results might indicate a protein turnover corresponding to the half-life of soluble liver proteins of 35 days in man. It has to be emphasized, however, that the protein synthesis rate determined in vitro in rat liver slices has been found to be 1/5 - 1/10 of the rate determined in vivo (68).

It might be questioned whether the liver tissue used in the protein synthesis experiments can be considered normal. Sunzel et al. (87) reported a higher triglyceride content (but no differences of phospholipids and cholesterol) in liver tissue from patients with gallstone disease than in patients with peptic ulcer. Nilsson & Scherstén (58), however, found no differences in the lipid synthesis pattern in liver slices from patients with diseases as different as gallstone disease, gastric disease, and renal calculus. So far, this seems to hold true also for the protein synthesis. This supports the assumption that the gallstone disease of the patients in the present study did not influence the results, especially not the comparison between the different patient groups.

Substrate incorporation into hepatic lipids and proteins in vitro in extrahepatic cholestasis (II).

In liver tissue from fifteen patients with extrahepatic cholestasis, we found an increased concentration ($P < 0.025$) of phosphoglycerides and pro-

teins compared with normolipidemic gallstone patients. The triglyceride concentration was equal while the glycogen concentration was decreased ($P \leq 0.05$) in these patients. The changed lipid and protein concentrations could not be ascribed to a change in the hepatic water content which was equal in the two groups.

The incorporation rate of glycerol into total phosphoglycerides ($P \leq 0.01$) as well as into the choline phosphoglycerides ($P \leq 0.05$) and ethanol-amine phosphoglycerides ($P \leq 0.01$) was increased in cholestatic liver tissue compared with controls. On the other hand, liver tissue from patients with cholestasis incorporated glycerol into triglycerides at the same rate as controls.

The incorporation rate of leucine into liver proteins was increased ($P \leq 0.0125$) in the cholestatic liver tissue, which also had a high protein content ($P \leq 0.025$) compared with the controls. These findings may be interpreted as indicating an enhanced synthesis of phosphoglycerides and proteins in cholestatic liver tissue.

This interpretation was supported by the higher concentrations of phosphoglycerides and proteins in the cholestatic liver slices. The final interpretation of these data should be made with caution, however, since the possibility of a changed specific activity in the immediate precursor pool can not be ruled out completely. The fact that the incorporation rate of glycerol into triglycerides was unchanged, concomitant with a significant increase in the incorporation rate into phosphoglycerides, does not support the hypothesis of a changed specific activity in the α-glycerophosphate pool. Moreover, the free leucine concentration was found to be almost equal (600-700 µmol/l) in cholestatic and normal liver tissue.

An enhanced synthesis of phosphoglycerides in liver tissue under cholestatic conditions may be explained by an increased bile acid concentration in the liver. Greim et al. (38) have shown that biliary obstruction causes an increased cholic acid concentration comparatively early, whereas chenodeoxycholic acid increased only when the cholestasis was protracted. A stimulating effect of bile acids on the lecithin synthesis seems to be well established in different species (3, 42, 59, 79, 81, 88). The mechanism of the effect of bile acids on the phospholipid synthesis is not known, however. We have previously shown (15) that in vitro addition of bile acids to incubations with liver slices caused a shunting of glycerol into lecithin synthesis pathways. This finding provided support for the hypothesis that bile acids, in some way, directly affect the hepatic synthesis of lecithin. This hypothesis is also supported by the finding of Kennedy & Weiss (48) that the lecithin synthesis in rat liver preparations was stimulated in the presence of detergents such as bile acids.

The mechanism behind the increased protein synthesis in cholestatic liver tissue is not easy to explain. An increased incorporation rate of amino acids into hepatic proteins in experimentally induced cholestasis in rats has been found (73). Morphometric analyses of electron micrographs of liver tissue from patients with cholestasis have shown an increased surface area of both the rough and smooth endoplasmic reticulum (37). This probably indicates a "hypertrophy" in that part of the cell which is mainly responsible for the protein synthesis.

Our finding of a significant correlation between the incorporation rate of glycerol into phosphoglycerides and the incorporation rate of leucine into proteins in cholestatic liver may suggest a common mechanism for the enhancement of both these processes. In one series of experiments with control liver slices, however, glycocholic acid or glycochenodeoxycholic acid was added to the incubation medium (final concentration 0.05 mmol/l). Neither one of these primary bile acids influenced the incorporation rate of leucine into proteins in contrast to the effect of the bile acids on the incorporation rate of glycerol into phosphoglycerides (79).

Another contributive mechanism to the enhanced incorporation of glycerol into fatty acids and leucine into proteins in cholestatic liver tissue has to be considered. Thus, the reduced form of nicotin-amide-adenine nucleotide monophosphate has been found to enhance the synthesis of amino acids and proteins (95). In a previous work, Scherstén et al. (78, 80) found that the ratio between reduced and oxidized nucleotides was considerably increased in cholestatic liver tissue. Thus, their findings are in line with those of the present study.

As pointed out in the introduction, extrahepatic cholestasis can be looked upon as a model system for dyslipoproteinemic conditions. Whether the changed plasma lipoprotein pattern in cholestasis is related to the changed liver tissue metabolism can not be unequivocally determined on the basis of the present results. It appears tempting, however, to assume that the increased plasma lecithin concentration in this condition may be caused at least partly by the enhanced hepatic synthesis. An additional cause may be a diminished lecithin secretion into bile. The appearance of the abnormal lipoprotein-X may be associated with such changes in the protein and phospholipid synthesis in the liver.

Effects of sucrose feeding in patients with prebeta hyperlipoproteinemia (III, IV).

Four patient groups were studied as described in detail in Papers III and IV. They are referred to as: non-sucrose-fed controls (n = 36), non-sucrose-fed patients with prebeta hyperlipoproteinemia (n = 8), sucrose-fed controls

(n = 15), and sucrose-fed patients with prebeta hyperlipoproteinemia (n = 13).

In the normolipoproteinemic patients, sucrose feeding produced an increase in the plasma triglycerides ($P < 0.001$), a transient decrease in the plasma cholesterol ($P < 0.005$), an increase in basal insulin ($P < 0.01$), concomitant with an increased lipoprotein lipase activity ($P < 0.01$). Only basal insulin increased significantly in the sucrose-fed patients with prebeta hyperlipoproteinemia.

Compared with non-sucrose-fed controls, the liver triglyceride content was increased in sucrose-fed controls ($P < 0.05$) as well as in non-sucrose-fed ($P < 0.05$) and sucrose-fed patients with prebeta hyperlipoproteinemia ($P < 0.05$). An increased incorporation rate of glycerol into hepatic triglycerides was found in the above-mentioned groups except for the sucrose-fed patients with prebeta hyperlipoproteinemia. The higher incorporation rate of precursors into triglycerides was taken as an indication of an increased hepatic triglyceride synthesis in the sucrose-fed controls and the non-sucrose-fed patients with prebeta hyperlipoproteinemia. The increased liver triglyceride content supports the validity of this finding. An additional fact which supported the finding was the significant correlation between the incorporation rate of precursors into hepatic triglycerides and the increment of plasma triglycerides on the seventh day of sucrose feeding in the sucrose-fed controls, as well as the significant correlation between the hepatic triglyceride synthesis and the plasma triglyceride concentration in the non-sucrose-fed patients with prebeta hyperlipoproteinemia.

In the sucrose-fed patients with prebeta hyperlipoproteinemia, the mean plasma triglycerides, as well as the mean incorporation rate of precursors into hepatic triglycerides, showed a wide range of values. This may be an expression of heterogeneity within this series of biochemically typed patients with prebeta hyperlipoproteinemia. It should be kept in mind that this patient group, in addition to comprising patients with Type IV, also included 6 patients with Type II B. Furthermore, it remains to be shown that patients with Type IV hyperlipoproteinemia constitute a metabolically homogenous population (70).

In the sucrose-fed patients with prebeta hyperlipoproteinemia, the mean of the incorporation rate of radioactive precursors into triglycerides was moderately increased but less than in the sucrose-fed controls. Caution should be exercised in the final interpretation of these data, however, since the possibility of a dilution of the label by an expanded precursor pool of α-glycerophosphate can not be completely ruled out. The facts that the incorporation rates of glycerol and fructose into triglycerides showed an increase, together with a highly significant decrease in the incorporation rate into phosphoglycerides, however, do not support the hypothesis of a dilution of the

immediate precursor pool. Furthermore, these findings are in agreement with conclusions drawn from other investigations in man (24, 29, 70).

The finding of a lower incorporation rate of glycerol, as well as of fructose, into hepatic choline phosphoglycerides in the sucrose-fed patients with prebeta hyperlipoproteinemia seems to be further supported by the lower liver content of phosphoglycerides in these patients. These data are also in agreement with our observation of a low choline phosphoglyceride concentration in the bile from the same patient group (16). The data also concur with the recent finding of Park et al. (65) that, in high carbohydrate-fed rats, the choline phosphoglyceride level in the rough microsome fraction of liver homogenates was significantly decreased and that the linoleic and arachidonic acids were virtually absent in the phospholipids of this fraction. Recently, Alling et al. (1) showed that sucrose feeding rapidly caused a significant decrease in the proportion of linoleic acid in liver, plasma and bile choline phosphoglycerides, indicating that sucrose feeding inhibits the cytidindiphosphate choline pathway (Kennedy's pathway) of the choline phosphoglyceride synthesis.

Thus, this decreased choline phosphoglyceride synthesis seems to be provoked by sucrose feeding, since non-sucrose-fed patients with prebeta hyperlipoproteinemia had a normal incorporation rate of precursors into hepatic glycerides as well as a normal hepatic phosphoglyceride content. Prebeta hyperlipoproteinemia is not characterized by low plasma phosphoglycerides or plasma choline phosphoglycerides (60), but the VLDL in Type IV have a lower specific density and a higher Sf value (4) and would, therefore, have a higher triglyceride/phosphoglyceride ratio than that of normolipoproteinemic subjects.

The low incorporation rates of glycerol and fructose into phosphoglycerides are most likely not an effect of the conditions chosen for the in vitro incubation. Neither an increase in the fatty acid amount in the system (58, 63) nor the use of another fatty acid substrate like oleic acid (43) or linoleic acid (15) would be expected to increase the incorporation into phosphoglycerides. The addition of insulin to the system would be expected to influence the incorporation into phosphoglycerides only to a moderate extent (91).

The incorporation rate of leucine into hepatic proteins was equal in non-sucrose-fed controls (n = 20) and non-sucrose-fed patients with prebeta hyperlipoproteinemia (n = 9). Three of the sucrose-fed controls (n = 4) incorporated leucine into proteins at a rate above the 95 per cent confidence level of that for non-sucrose-fed controls. This may indicate an insulin effect on the hepatic protein synthesis capacity (I, 51), which has been found also after long-term glucose feeding in rats (23). No change in the incorporation rate of leucine into hepatic proteins in sucrose-fed patients with prebeta

hyperlipoproteinemia was found in the present investigation. In spite of the limited number of subjects studied, the question must be raised if this difference was founded on a changed insulin sensitivity or on a different response in the liver of the sucrose-fed patients with hyperlipoproteinemia. It appears to be well established that plasma insulin stimulates the adipose tissue lipoprotein lipase activity and in this way facilitates the removal of VLDL. Both in controls and in patients with prebeta hyperlipoproteinemia, sucrose feeding caused an increase in the basal insulin level, but the adipose tissue lipoprotein lipase increased only in controls. This finding may support the hypothesis of a changed sensitivity or response to insulin in prebeta hyperlipoproteinemia.

So far, the findings in the present study agree with the reports of Reaven et al. (72) and Nikkilä & Kekki (55) that the triglyceride turnover is above normal in patients with endogenous hyperlipoproteinemia. Based on their own observations, Nikkilä & Kekki (55) suggested that the primary defect in most cases of Type IV endogenous hyperlipoproteinemia is an increased inflow transport of plasma triglycerides. Sucrose-fed patients with prebeta hyperlipoproteinemia showed a decreased fractional elimination rate ($P < 0.05$) of exogenous fat without changes in lipoprotein lipase activity, also indicating an insufficient removal capacity for plasma triglycerides. In the sucrose-fed controls, on the other hand, we found a significantly increased lipoprotein lipase activity together with a change from an increase toward normal initial plasma triglyceride levels which indicated an adequately clearing function of VLDL. Thus, an increased transfer of triglycerides from the liver into plasma causes a greater concentration increase in subjects with inherently "weak" removal systems. Obviously, there are indications of an increased production, as well as of a defect removal, of plasma VLDL in patients with prebeta hyperlipoproteinemia.

SUMMARY

1. Liver tissue from normolipidemic gallstone patients older than 60 years incorporated leucine into hepatic proteins at a higher rate than liver tissue from younger individuals, indicating a higher protein turnover rate in the older patients.

2. Cholestatic liver tissue contained more proteins and phosphoglycerides, and incorporated precursors into proteins and phosphoglycerides at a higher rate than presumably normal liver tissue. The incorporation rate of glycerol into hepatic phosphoglycerides correlated significantly with the plasma phosphoglyceride concentration.

3. Liver tissue from patients with prebeta hyperlipoproteinemia on an ordinary diet contained more triglycerides and incorporated glycerol into triglycerides at a higher rate than liver tissue from normolipidemic gallstone patients. The incorporation rate of precursors into hepatic triglycerides correlated significantly with the plasma triglyceride concentration, indicating an increased hepatic triglyceride synthesis rate.

4. After sucrose feeding for 14 days, liver tissue from patients with prebeta hyperlipoproteinemia had a lower content of phosphoglycerides and incorporated precursors into phosphoglycerides at a lower rate compared with non-sucrose-fed patients with prebeta hyperlipoproteinemia, indicating a phosphoglyceride synthesis defect provoked by the sucrose feeding.

5. Sucrose-fed controls showed an increased incorporation rate of glycerol and fructose into hepatic triglycerides in vitro, which correlated with the increase in the plasma triglyceride concentration indicating an increased triglyceride synthesis rate.

6. The fractional elimination rate of exogenous fat decreased after sucrose feeding in controls as well as in patients with prebeta hyperlipoproteinemia, indicating a removal defect for at least exogenous fat.

7. The adipose tissue lipoprotein lipase activity was increased after sucrose feeding in controls and was inversely correlated to the plasma triglyceride concentration in controls as well as in patients with prebeta hyperlipoproteinemia.

ACKNOWLEDGEMENTS

My sincere thanks to:

Associate professor Tore Scherstén, M.D., who initiated the present study and has been my teacher and co-worker.

Professor Lars Werkö, M.D., and professor Ragnar Romanus, M.D., who have given me excellent laboratory facilities and has followed the investigations with interest.

Associate professor Anders Gustafson, M.D., for help with all serum lipid electrophoresis and for fruitful discussions.

Associate professor Rudolf Jagenburg, M.D., for help with the amino acid analyses.

Mrs Ingela Jonson, Mrs Barbro Jilderos, Miss Ingela Östrand and Mrs Margareta Jonsson for scilful technical assistance.

Miss Lena Andersson, Mrs Gunilla Pacsay and Mrs Kerstin Grahn for excellent secreterial help.

Mr Hugo Svensson for his scilful making of all the figures.

Mrs Erika Johansson for excellent photographing.

My co-workers and to all colleagues at the Surgical Departments, Sahlgrenska sjukhuset, who have willingly performed liver biopsies.

AB ASTRA, Södertälje for economical support and for supplying amino acid solutions.

This investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases, the Swedish Medical Research Council (project No 536), the Swedish Cancer Society (project No 93) and from the Medical Faculty, University of Göteborg.

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