The effect of a rat plasma high-density lipoprotein subfraction on the synthesis of bile salts by rat hepatocyte monolayers

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Received 26 October 1984

The effect of a rat high-density lipoprotein subfraction (HDL_2) on the synthesis of bile salts by rat hepatocyte monolayers prepared from rats fed a diet containing cholestyramine, was investigated. The synthesis of bile salts as measured by radioimmunoassay of conjugated cholic, chenodeoxycholic and β-muricholic acids was significantly increased when hepatocytes were incubated with a physiological concentration (500 μg HDL_2 protein · ml^{-1}) of HDL_2.

Rat hepatocyte monolayer *Bile salt synthesis* *Cholestyramine* *High-density lipoprotein*

1. INTRODUCTION

It has been suggested that of the plasma lipoproteins, HDL provides a vehicle whereby excess cholesterol from the peripheral tissues can be delivered to the liver for its ultimate removal from the body [1,2]. This could be achieved by either degradation of cholesterol to bile acids or by direct secretion of the unmodified sterol into the bile. Whilst evidence has shown that the liver is a major site for the catabolism of HDL [3] no evidence has been reported to indicate that HDL can stimulate the degradation of cholesterol to bile salts. Recently it was reported that of the rat plasma lipoproteins, a fraction of density < 1.02 g/ml increased the synthesis of bile acids by rat hepatocytes [4]. HDL was found to have no effect.

Interruption of the enterohepatic circulation by feeding the bile salt sequestrant cholestyramine is known to increase the synthesis of bile salts both in vivo [5] and in isolated hepatocytes [6,7]. It has also been shown, in humans and rabbits, that feeding cholestyramine results in an increase in the uptake of LDL from plasma [8,9]. These results suggest that the uptake of lipoproteins by the liver and the synthesis of bile salts may be interrelated. However, it has been reported that dietary cholestyramine does not affect the uptake of plasma lipoproteins in rats [10].

Rat plasma HDL can be subdivided into two major fractions; a lighter HDL_1 and a denser HDL_2 fraction [11]. Here, we show that incubation of rat hepatocyte monolayers prepared from animals fed cholestyramine with a subfraction of rat HDL increases the synthesis of bile salts.

2. MATERIALS AND METHODS

2.1. *Materials*

Collagenase, galactose, gentamycin sulphate and insulin were obtained from Sigma (London). Dulbecco's modified Eagle's medium and streptomycin/penicillin were from GIBCO Europe (Paisley, Scotland); non-essential amino acids and foetal calf serum were from Flow Laboratories (Irvine, Scotland). Foetal calf serum was heat-

* Professor G.S. Boyd died on 10th January 1983

Abbreviation: HDL, high-density lipoprotein

Published by Elsevier Science Publishers B.V.
00145793/85/$3.30

inactivated by incubating for 30 min at 56°C. Cholestyramine ('Cuemid') was a product of Merck, Sharp and Dohme (West Point, Philadelphia, PA); 'Percoll' was obtained from Pharmacia (Great Britain).

2.2. *Preparation of rat HDL_2*

Blood was obtained from female Wistar rats (200–400 g) maintained on a standard pellet diet and collected in 10 ml polypropylene tubes containing 40 mg EDTA. Blood cells were separated from plasma by low-speed centrifugation. HDL_2 was then prepared as in [11].

2.3. *Preparation of rat hepatocyte monolayers*

Female Wistar rats (150–250 g) were maintained on a diet consisting of 70% wholemeal flour, 25% skimmed milk powder and 5% dried yeast supplemented with 4% cholestyramine. Animals were allowed access to food and water ad libitum. Hepatocytes were prepared as in [12] except that hyaluronidase was omitted from the appropriate perfusion medium and 0.5 mM calcium was added. Following isolation, the hepatocytes were subjected to centrifugation on a 0–70% Percoll density gradient for 15 min at $1500 \times g$ to separate non-viable cells from viable cells. The viability of hepatocytes obtained was routinely greater than 95% as assessed by the exclusion of Trypan blue. Hepatocytes were maintained in monolayers for up to 24 h. Attachment of hepatocytes to petri dishes was facilitated by incubation of cells for 2 h in Dulbecco's modified Eagle's medium supplemented with 0.4 mM ornithine hydrochloride, non-essential amino acids, gentamycin sulphate (100 mg/ml), penicillin/streptomycin (10000 IU/l; 10000 mg/l), nystatin (20000 U/l) and galactose (1 g/l) and containing 15% (v/v) foetal calf serum and insulin (100 U/l). Following the adhesion of the cells to petri dishes, the hepatocytes were maintained in the supplemented Dulbecco's modified Eagles medium without serum and insulin.

2.4. *Radioimmunoassay*

Conjugated cholic, chenodeoxycholic and β-muricholic acids were determined using radioimmunoassays [13–15]. The rabbit antiserum used in the β-muricholic acid assay differed from that in [15] in that it maintained 100% cross-reactivity between the tauro- and glycoconjugates. The bile salts detected at 0 h represent cell-associated levels following adhesion of hepatocytes to petri dishes. The cellular bile salt content was determined after overnight extraction with 1 M sodium hydroxide [6]. Samples were neutralised with hydrochloric acid prior to assay. Bile salt content of the medium was measured directly. The total amount of the individual bile salts synthesised by the rat hepatocyte monolayers was determined by addition of the amount present in the medium to that in the cells.

2.5. *Determination of protein*

Protein was determined as in [16] using bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

Rat hepatocytes were maintained in monolayers for up to 24 h. The synthesis of conjugated cholic, conjugated chenodeoxycholic and conjugated β-muricholic acids were subsequently determined. These three bile salts have been reported to represent at least 95% of the total bile salts synthesised by isolated hepatocytes [7]. It is therefore likely that the measurement of conjugated cholic, conjugated chenodeoxycholic and conjugated β-muricholic acids is representative of any changes which may occur in the synthesis of bile salts. For clarity, the total synthesis of bile salts is represented by the addition of the mass of the three bile acid conjugates measured.

The total synthesis of bile salts by rat hepatocyte monolayers prepared from rats fed the diet supplemented with 4% cholestyramine compared with hepatocyte monolayers prepared from rats fed a control diet, was significantly increased (fig.1). This is consistent with the known effects of cholestyramine [5–7].

When hepatocyte monolayers prepared from rats fed cholestyramine were incubated with HDL_2 (500 μg HDL_2 protein/ml) the total synthesis of the bile salts measured was significantly increased (fig.2). These results indicate that in these cells HDL_2 can deliver cholesterol to the liver and that this cholesterol enters the substrate pool for the synthesis of bile salts.

When the experiment was repeated using hepatocytes obtained from rats fed a control diet, HDL_2 had no effect on the synthesis of bile salts (not shown). This latter finding is consistent with

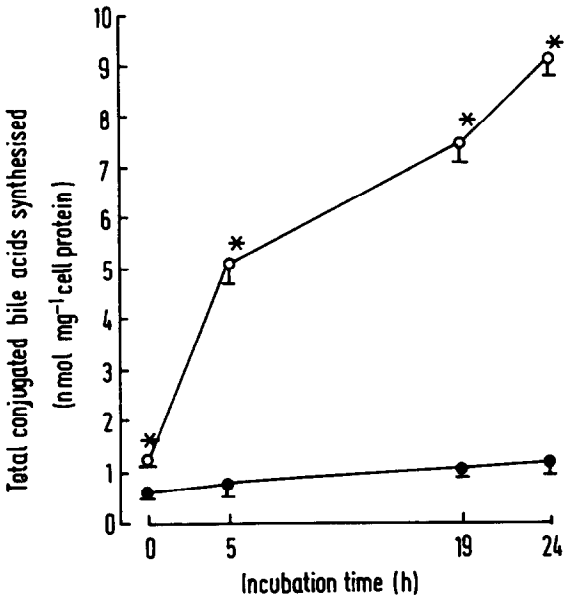

Fig.1. The effect of diet on the synthesis of bile salts by rat hepatocyte monolayers. Hepatocyte monolayers were prepared from rats fed a soft diet (●—●) or the soft diet + 4% cholestyramine (○—○). Bile salts detected at 0 h represent cell-associated levels following adhesion of hepatocytes to petri dishes. Each point represents the mean of duplicate determinations from hepatocytes obtained from 4 rats. Significance limits, * $P < 0.05$. Error bars show ± SD.

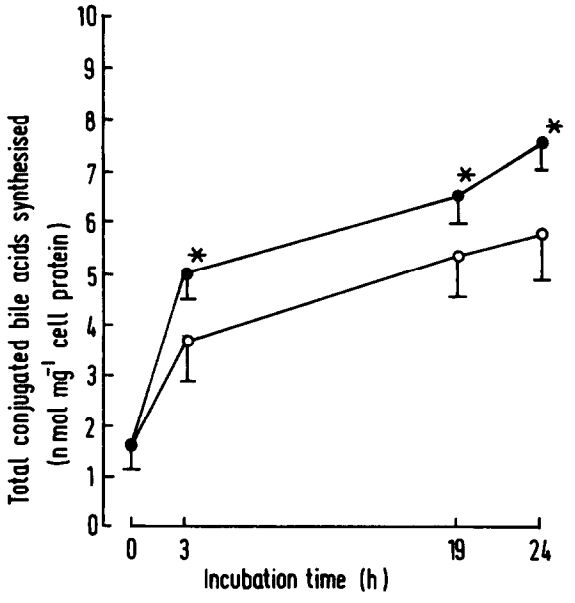

Fig.2. Effect of HDL_2 on bile salt synthesis by rat hepatocyte monolayers. Hepatocytes isolated from rats fed the soft diet + 4% cholestyramine were maintained in monolayers in the absence (○—○) or presence (●—●) of HDL_2 (500 μg HDL_2 protein/ml) for the indicated time periods. Bile salts detected at 0 h represent cell-associated levels following adhesion of hepatocytes to petri dishes. Each point represents the mean of duplicate determinations from hepatocytes obtained from 6 rats. Significance limits, * $P < 0.05$. Error bars show ± SD.

the work in [4].

The observed increase in the synthesis of bile salts by hepatocytes in the presence of HDL_2 may reflect the nutritional status of the rats used for the preparation of the hepatocytes. Feeding cholestyramine results in an increase in the synthesis of bile salts [5–7], an increase in the uptake of lipoproteins by the liver in certain species [8,9] but not the rat [10], and also stimulates hepatic cholesterogenesis [17]. Feeding cholestyramine therefore results in an increase in the influx of cholesterol to the metabolic pool of cholesterol within the hepatocyte. The contribution of plasma cholesterol *vs* cholesterol derived from de novo synthesis to this increase is likely to vary from species to species [8–10]. Following utilization of the preformed pool of cholesterol (i.e., cholesterol derived from the plasma and equilibrated with newly synthesised cholesterol) hepatocytes incubated in medium not containing HDL_2 are likely to obtain the cholesterol utilized for the synthesis of bile salts entirely from de novo synthesis. It is quite possible that the substrate derived from newly synthesised cholesterol does not fill the capacity of the rate-limiting enzyme of the pathway for the synthesis of bile salts in those cells prepared from animals fed cholestyramine. However, addition of HDL_2 to the culture medium provides an additional source of cholesterol and, on entering the hepatocyte, this lipoprotein-derived sterol can be degraded to bile salts. The increase in the synthesis of bile salts observed in the presence of HDL_2 (fig.2) may therefore reflect the mass of plasma cholesterol normally degraded to bile salts in vivo. It appears likely that hepatocytes obtained from rats fed cholestyramine require an exogenous source of cholesterol for optimum rates of bile salt synthesis.

The ratio of conjugated cholic acid:conjugated

Table 1

Effect of rat HDL_2 on the ratio of conjugated cholic acid:conjugated chenodeoxycholic + β-muricholic acids and the ratio of conjugated chenodeoxycholic acid:conjugated β-muricholic acid, as a measure of 12 α-hydroxylation and 6 β-hydroxylation, respectively

Time (h)	Conjugated cholic acid/ conjugated chenodeoxycholic + β-muricholic acids		Conjugated chenodeoxycholic acid/conjugated β-muricholic acid	
	Control	+ HDL_2	Control	+ HDL_2
0	1.1 ± 0.3	1.1 ± 0.3	0.63 ± 0.3	0.63 ± 0.3
3	1.35 ± 0.6	1.12 ± 0.3	0.19 ± 0.06	0.22 ± 0.07
19	1.27 ± 0.5	1.35 ± 0.7	0.08 ± 0.03	0.07 ± 0.03
24	1.34 ± 0.4	1.29 ± 0.4	0.05 ± 0.02	0.04 ± 0.01

Results are the mean ± SD of results obtained from 6 rats

chenodeoxycholic acid + conjugated β-muricholic acids and the ratio of conjugated chenodeoxycholic acid:conjugated β-muricholic acid were also determined. These ratios provide a good indication of the rates of 12 α-hydroxylation and 6 β-hydroxylation, respectively. Twelve α-hydroxylation controls the ratio of conjugated cholic acid to conjugated chenodeoxycholic acid and its metabolite β-muricholic acid whilst 6 β-hydroxylation provides an indication of the rate of conversion of chenodeoxycholic acid to β-muricholic acid. HDL_2 was found to have no effect on these ratios (table 1). The results suggest that HDL_2-cholesterol enters a common precursor pool which provides cholesterol for the synthesis of the major bile acid conjugates measured.

These experiments provide the first indication that HDL can stimulate the synthesis of bile salts by hepatocytes. This increase is likely to be a reflection of the nutritional status of the animal and may indicate that hepatocytes isolated from rats fed cholestyramine are deficient in substrate for the synthesis of bile salts.

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