INTERACTION OF HUMAN SERUM HIGH DENSITY LIPOPROTEINS WITH TRIGLYCERIDES IN VITRO

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1. Introduction

Almost twenty years ago Korn [1] put forward a hypothesis that between chylomicrons and α-lipoproteins there exists a recycling system which is responsible for the transport of a part of triglycerides in the human body.

So far this hypothesis has neither been refuted nor supported by direct experimental evidence. There is, however, some indirect evidence in favor of Korn's concept: it has been established, for example, that following injection of heparin very low density lipoproteins (VLDL) in human plasma decrease and high density lipoproteins (HDL) increase [2]. Experiments in which the distribution of labelled VLDL and HDL apoproteins was studied following intravenous administration showed that transformation of VLDL into lipoproteins of higher density exists both in man and rat [3–6]. However, only some vague hints at the possibility of re-utilization of VLDL apoproteins have been given [6].

In this communication experimental evidence is presented on the formation of VLDL from human HDL and triglycerides in vitro.

2. Materials and methods

Acrylamide, methylenebisacrylamide and TEMED were purchased from Canalco (USA), Sephadex G-200 from Pharmacia (Sweden), pure Triolein from Calbiochem (USA) and ^{131}I-labelled Triolein from Mallinckrodt (USA).

2.1. *Purification of human HDL*

Human HDL were isolated from 50 ml of fresh pooled serum collected from fasted male and female subjects aged 30–50 years by precipitation with sodium phosphotungstate and $MgCl_2$ according to the method of Burstein and Scholnick [7]. After precipitation the HDL fraction was dissolved in 10 ml 0.05M Tris–HCl buffer, pH 7.4, containing 0.15 M NaCl. This solution was dialysed against the same buffer for 12 hr at 4°C. Eight ml of the dialyzed HDL solution were subjected to gel filtration on a Sephadex G-200 column (2.5 X 85 cm) equilibrated and eluted (14 ml/h) with the same buffer. Absorption at 280 nm in the eluate was monitored. Four-ml fractions were collected and in each lipoproteins were analyzed by disc electrophoresis. Five adjacent fractions with the greatest amount of HDL were pooled and about 20 ml of final HDL solution was obtained. In the pooled HDL solution protein [8], total lipid [9], triglyceride [10], total cholesterol [11] and phospholipid [12] determinations were always performed. The purity of HDL was checked by polyacrylamide electrophoresis, immunoelectrophoresis and immunodiffusion.

Purified HDL were also delipidated with ethanol-ether (3:1) mixture to obtain apoproteins [13].

2.2. *Electrophoretic procedures*

HDL were analyzed by electrophoresis on 7.5% polyacrylamide gel [14] and apoproteins on 7.5% polyacrylamide gel containing 8 M urea. Staining was performed by 1% amido black in 7% acetic acid and destaining in 7% acetic acid. The products of the

interaction of HDL with triolein were analyzed by electrophoresis on discontinuous polyacrylamide gel gradient after prestaining with 1% Sudan Black B. The gels were prepared by the method of Wollenweber and Kahlke [15], so that each gel consisted of 15, 12, 4.1, 3.75, 3.1 and 2.8% acrylamide segments, counting from the bottom to the top. Electrophoresis was performed in a Shandon apparatus (Great Britain) for 8 tubes, at pH 8.3 (0.05 M Tris–glycine buffer), and constant power of 4 mA per tube. Immediately after separation was completed (30–40 min) the intensity of lipoprotein fractions was measured by a Kipp and Zonen (Holland) DD2 densitometer and BC1 digital integrator. When radiolabelled ^{131}I-Triolein was used lipoprotein fractions were sliced off and their radioactivity was measured in a Mark II (Nuclear Chicago) liquid scintillation counter using gamma vials. Immunoelectrophoresis of HDL and serum was performed according to the method of Ouchterloney [16].

2.3. *Purification of lipoprotein lipase*

Lipoprotein lipase was purified from rat (Fischer strain) epididymal adipose tissue by the method of Greten [17].

2.4. *HDL-Triolein interaction*

HDL–Triolein complexes were formed from pure or ^{131}I-labelled Triolein and purified HDL in various HDL: Triolein ratios. The triolein added into tubes was always dissolved in benzene. After removing benzene in a stream of nitrogen, 0.3 ml of HDL solution was added and the sample was vortexed for 5 min (with Vortex K-550-GE, Scientific Industries, Inc., USA) at 25°C at maximal speed. The Triolein–HDL mixture was then analyzed by disc electrophoresis on gel gradients as described above and by electron microscopy. In the electron microscopic examination a drop of the reaction mixture was mixed with a drop of 2% sodium phosphotungstate on top of a copper gird and immediately examined with a Siemens Elmiskop IA.

On several occasions some of the HDL–triglyceride mixtures (0.2 ml) were incubated for 15, 30 and 60 min at pH 8.6, 37°C with purified lipoprotein lipase (0.2 ml). The effect of lipoprotein lipase was studied by electrophoresis on gel gradients and by free fatty acid release estimation [18]. In some experiments HDL apoproteins were also vortexed with various amounts of triglycerides (from 0.5 to 2.0 Triolein: protein ratio).

3. Results

The properties of HDL purified by precipitation with sodium phosphotungstate and $MgCl_2$, and gel filtration on Sephadex G-200 are presented in table 1. The apparent molecular weight calculated from the HDL elution volume is about 160 000.

Table 1
Concentrations of proteins and lipids (mg/ml) in purified HDL solution

Proteins	Total lipids	Total cholesterol	Triglycerides	Phospholipids
1.74	1.91	0.18	0.18	0.91
± 0.28	± 0.37	± 0.01	± 0.04	± 0.07
(11)	(11)	(11)	(7)	(7)
48%	52%	5%	5%	25%
3½	3½	3½	3½	3½

Mean ± standard error.
Number of samples in parentheses.

It was ascertained by disc electrophoresis on 7.5% acrylamide gels that purified HDL preparation contained only one, rather diffuse protein band (fig. 1E). Immunoelectrophoresis revealed only one precipitation line, which corresponds to the α_1 lipoprotein precipitation line (fig. 1G). Immunodiffusion showed no traces of albumin (fig. 1H). Delipidated HDL displayed apoAI, apoAII and apoC apoproteins (fig. 1F). On the discontinuous gel gradient (fig. 1C) normal human serum showed 5 lipoprotein bands, while HDL only one band entering the 12%-gel (fig. 1D).

When the HDL solution was Vortexed for 5 min with Triolein, an emulsion was formed in which several anode migrating lipoprotein fractions could be detected by electrophoresis on discontinuous polyacrylamide gel gradient (fig. 2). These new lipoproteins contained radioactivity when ^{131}I-Triolein was used. They had the electrophoretic mobility of VLDL. We call them $VLDL_1$, $VLDL_2$, $VLDL_3$ and $VLDL_4$, according to their migration distance on discontinuous

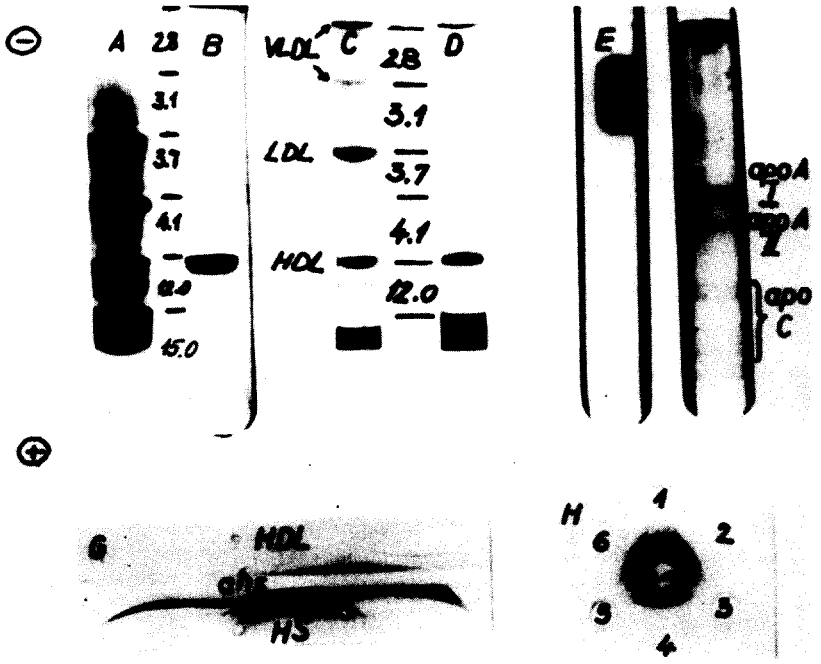

Fig. 1. Results of HDL purification. A: Electropherogram of pooled human serum proteins on discontinuous polyacrylamide gel gradient (stained with Amido Black). 11 fractions are separated. B: Electropherogram of purified HDL on discontinuous polyacrylamide gel gradient (stained with Amido Black). Only one fraction is visible at the level of 12% gel. The numbers correspond to the percentage of acrylamide in each gel segment. C: Electropherogram of pooled human serum lipoproteins (prestained with Sudan Balck B) on discontinuous polyacrylamide gel gradient. VLDL are at the start and at the level of 3.1% gel, LDL in 3.75% gel and HDL is entering 12% gel. Acrylamide % of single segments is given between C and D. D: Electropherogram of the purified HDL preparation (prestained with Sudan black B) on discontinous polyacrylamide gel gradient. Only HDL are present. E: Electropherogram of the purified HDL preparation on 7.5% polyacrylamide gel (stained with Amido Black). One protein fraction in the upper third of the gel is present. F: Electropherogram of totally delipidated HDL apoproteins on 7.5% polyacrylamide gel (stained with Amido Black). ApoAI, apoAII and apoC apoproteins are present. G: Immunoelectropherogram of purified HDL and pooled human serum (HS). Only one precipitation line, which corresponds to α_1 lipoprotein, is present in the HDL preparation. ahs = rabbit antihuman serum. H: Immunodiffusion of purified HDL (1,3,5) and human albumin (2,4,6) against rabbit antialbumin serum (aa). The HDL preparation displayed no precipitation lines.

gel gradient (fig. 2). Electron microscopy (fig. 4) revealed, besides some intact HDL molecules, the presence of spherical particles of various diameters (300–3800 Å). The formation of various VLDL types, depending on the amount of triglyceride in the mixture is presented in fig. 3. The dependence of the amount of ^{131}I-Triolein incorporated into $VLDL_2$ and $VLDL_3$ upon the HDL/triolein/w/w) ratio in the reaction mixture is presented in fig. 5. At 1.5 HDL/Triolein ratio about 60% of the Triolein amount is incorporated into $VLDL_2$ and $VLDL_3$.

As evidenced by the changes in the electrophoretic pattern (fig. 6) and free fatty acids release, which is 0.6 to 0.8 μmoles FFA/mg protein/hr, lipoprotein lipase from rat epididymal adipose tissue can degrade VLDL fractions formed in vitro. In the HDL apoproteins–Triolein mixture formation of VLDL was not found.

4. Discussion

It is possible to form triglyceride-rich lipoproteins from purified human HDL and triglyceride in vitro. Their electrophoretic mobility and appearance under electron microscope are similar to VLDL. They can also serve as lipoprotein lipase substrate. It is quite obvious that the separation of these in vitro-formed

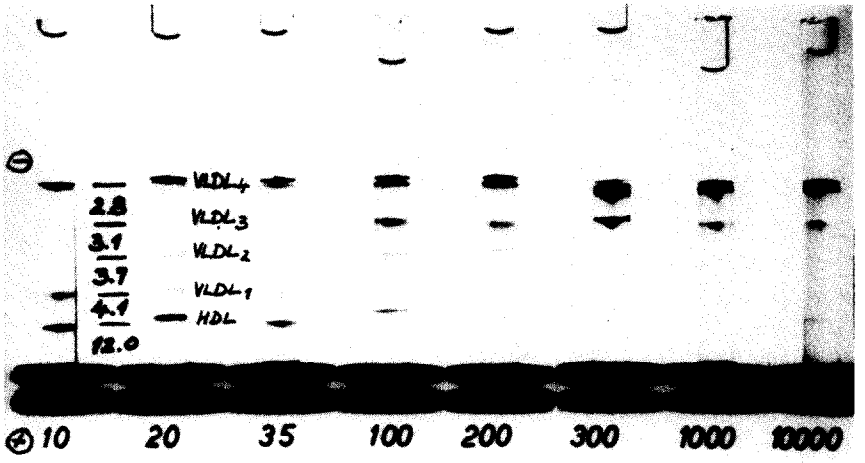

Fig. 2. Electropherograms of the reactions mixtures containing 100 μg HDL and various amounts of Triolein (10 μg–10 mg) on discontinuous polyacrylamide gel gradient. The percentages of acrylamide in each gel segment are shown between the first 2 gels and the amounts of Triolein (μg) in the reaction mixtures are given under each gel. The positions of single lipoprotein fractions are noted between the second and third gel. The characteristic changes in the intensity of four VLDL and HDL bands with increasing amounts Triolein are evident.

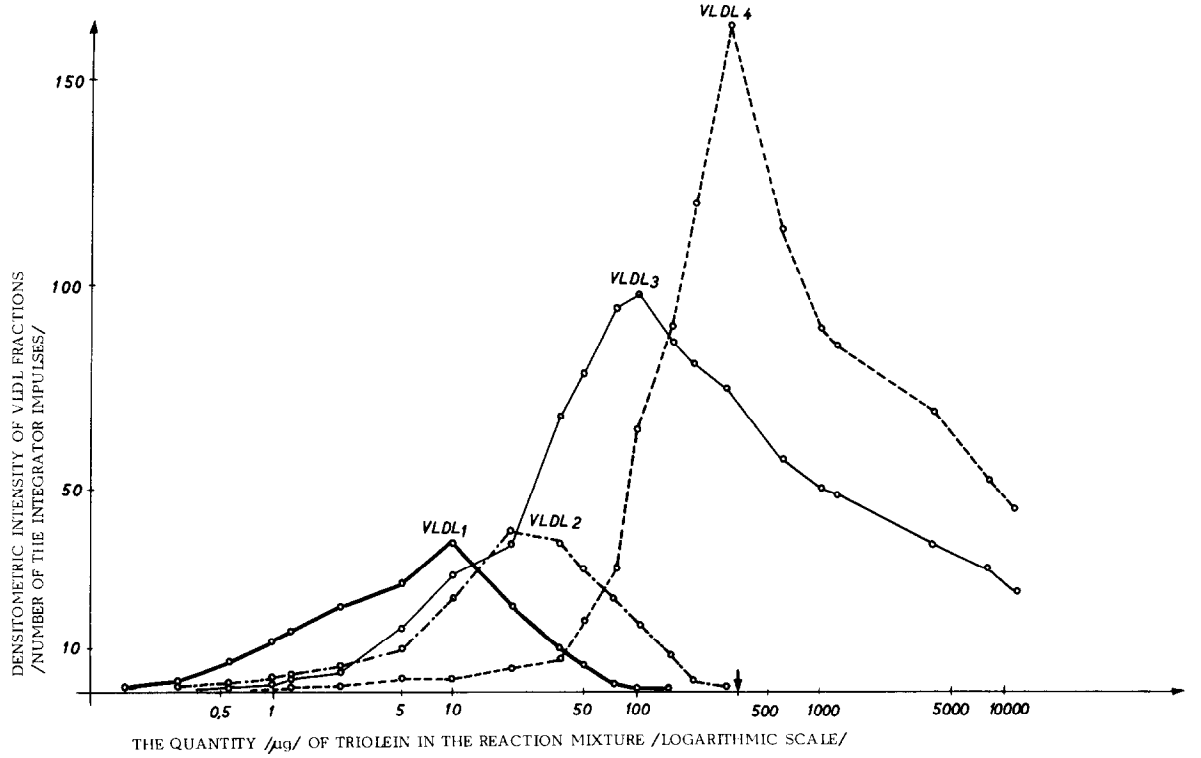

Fig. 3. Dependence of the densitometric intensity of single VLDL fractions upon the amount of triolein in the HDL–Triolein reaction mixture. Each reaction mixture had a volume of 0.5 ml and contained 100 μg HDL. The arrow points to the amount of Triolein at which total VLDL intensity was the greatest.

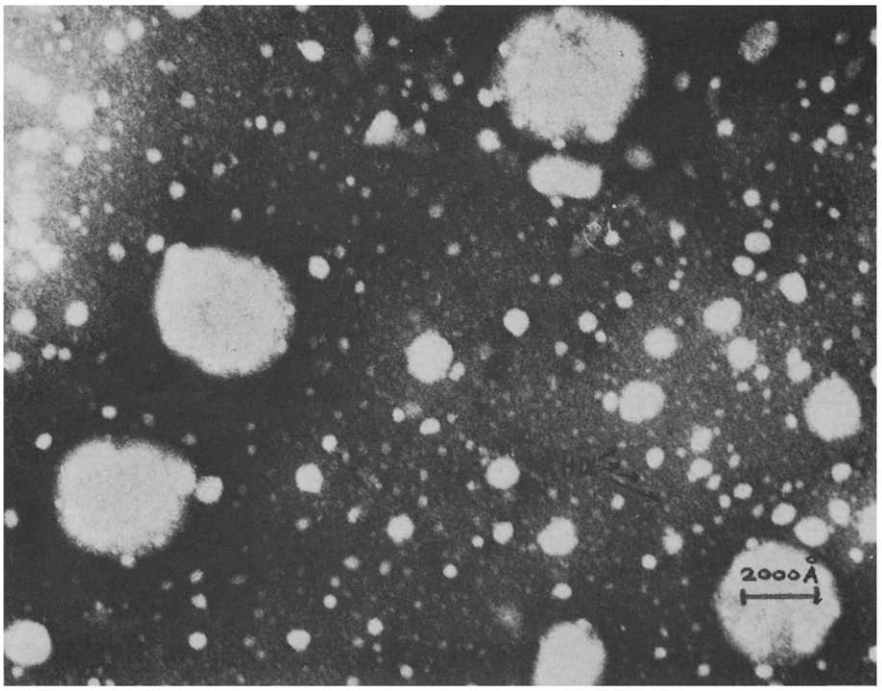

Fig. 4. Electron micrograph of the reaction mixture containing 100 μg HDL and 300 μg Triolein. Spherical particles of various sizes (300–3800 Å) and HDL molecules are visible. (Negative staining with 2% sodium tungstate, 50 000 × magnification.)

VLDL into 4 fractions is rather artificial, because it is obtained on discontinuous gel gradient. It is most probable that, in fact, a continuous spectrum of VLDL of various sizes is formed, i.e., that the size of VLDLs that predominate in the reaction mixture changes with the HDL/triglyceride ratio.

It is interesting that the total amount of triglycerides incorporated into all fractions of VLDL is the greatest at the triglyceride concentration which is on the borderline of the emulsive capacity of the amount of HDL in the reaction mixture, i.e., at 0.3 HDL/triglyceride ratio, (w/w) (fig. 3, arrow). Further increase in the concentration of triglycerides elicits a change in the macroscopic appearance of the emulsion: on the surface droplets of non-emulsified triglycerides, whose size increases with concentration, will be formed. This change is paralleled by a decrease in the intensity of VLDL fractions (figs. 2 and 3).

In studies of lipoprotein lipase activity evidence has accumulated that serum, i.e., serum lipoproteins can interact in vitro with various triglyceride emulsions [19–21]. Through this 'activation' triglycerides are made susceptible to hydrolysis by lipoprotein lipase. In this sense HDL and VLDL or their apoproteins have been shown to be the most potent 'activators'. However, little is known about the mechanism of the interaction between triglycerides and lipoproteins or apoproteins. Whayne [22] only hypothesized that in some way HDL change the surface of triglyceride particles.

The data presented here cannot fully explain the mechanism of formation of VLDL in vitro, but it is possible that triglycerides, for example, interact with fragments of HDL which, in some way, become dissociated during the reaction. Apoprotein C, for example, can be easily detached from the HDL molecule [23]. In fact, apoCII appears to be incorporated into the VLDL formed in vitro, because this apoprotein is the only activator of rat adipose tissue lipoprotein lipase [24] which we proved to degrade VLDL formed in vitro. We do not know as yet what other apoproteins are present in the VLDL formed in vitro, but it is obvious that besides apoproteins some lipid components of HDL, most probably phospholipids, are also neces-

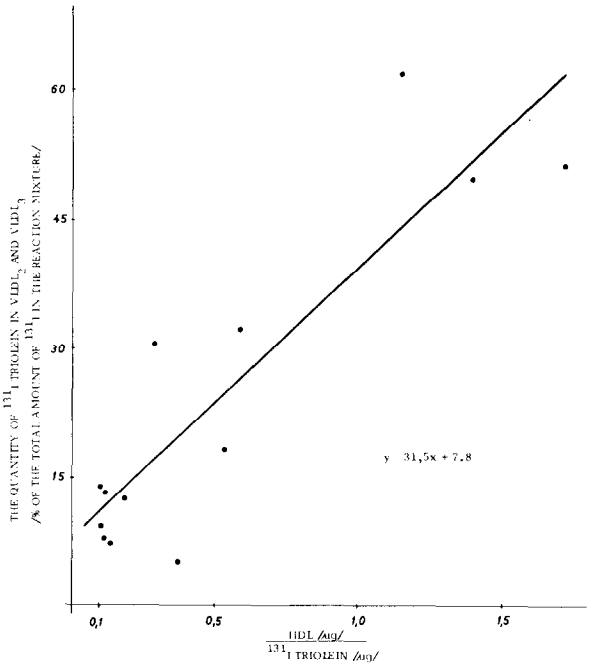

Fig. 5. Correlation between the amount of ^{131}I-Triolein incorporated into $VLDL_2$ and $VLDL_3$ and the HDL/^{131}I-Triolein ratio (w/w). The maximal incorporation of ^{131}I-Triolein into $VLDL_3$/about 60% of the total amount of Triolein in the reaction mixture/ is at the 1.1 ratio.

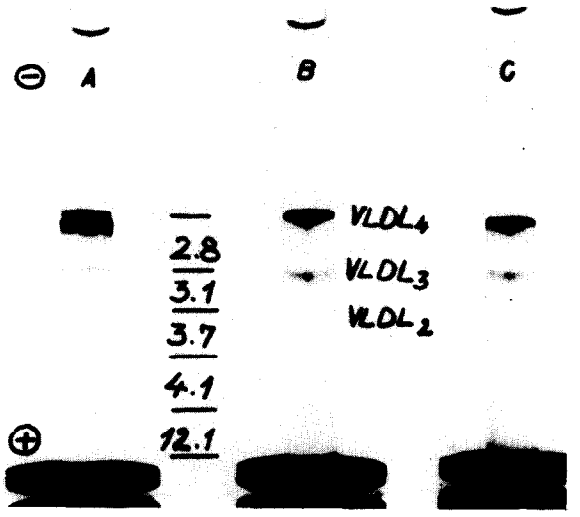

Fig. 6. Electropherograms of lipoproteins in the incubation mixture containing $VLDL_3$ and $VLDL_4$ and purified lipoprotein lipase from rat adipose tissue on discontinuous polyacrylamide gel gradient; (A) at the beginning of incubation; (B) after 30 min incubation and (C) after 60 min incubation. The decrease in the intensity of the $VLDL_4$ band is paralleled by an increase in the intensity of the $VLDL_3$ and $VLDL_2$ bands (the percentage of each polyacrylamide segment is noted between gels A and B).

sary for the formation of VLDL, because delipidated HDL apoproteins alone cannot form VLDL fractions with triglycerides.

In any event, our results indicate the possible existence of a recycling system between VLDL and HDL in vivo and suggest that further research should be directed to the study of the possible role of this system in the transport of triglycerides.

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