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IN VITRO SYNTHESIS OF APO-A-IV AND APO-C BY LIVER AND INTESTINAL mRNAs FROM LEAN AND OBESE ZUCKER RATS

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SUMMARY: The relative content and expression of mRNA coding for apolipoproteins A-IV and C in liver and intestinal cells have been studied in obese hyperlipemic (fa/fa) and lean (Fa/-) Zucker rats. The RNA were translated in a rabbit reticulocyte lysate system using ^{35}S-methionine as label. Apo-A-IV and apo-C were immunoprecipitated with specific antibodies and characterized by electrophoresis and autoradiography. The content and expression of mRNA specific for apo-A-IV and apo-C was much higher and the liver of obese than of lean rats. The apo-A-IV and apo-C synthetic capacity of intestinal cells from fa/fa and Fa cells from fa/fa and Fa rats was similar suggesting that the lipoprotein overproduction, already described in obese Zucker rats, is mainly from hepatic origin.

The genetically obese Zucker rat (1) provides an interesting model to investigate the mechanisms of hormonal and nutritional control of liver metabolism. Indeed, this form of obesity, which is linked to the recessive mutant gene "fa" is characterized by hyperinsulinemia and hypoglucagonemia as well as by several liver metabolic abnormalities. One of the most prominent characteristics of the genetically obese Zucker rat is the markedly elevated level of plasma triglyceride (2). Both VLDL and chylomicron remnant particles (3,4) accumulate in the circulation of these animals. Previous studies have implicated an increased hepatic production of very low density lipoproteins (VLDL) in the development of this hypertriglyceridemic state (5,6).

The contribution of intestinal secretion of lipoproteins has not been as yet documented although the increased concentration of chylomicron remnants suggest an inbalance between chylomicron production

Abbreviations :
VLDL : very low density lipoproteins ; LDL : low density lipoproteins ; HDL : high density lipoproteins.

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and catabolism. Recently, an increase in the mass of apolipoprotein B secreted by the perfused livers from obese rats with a three-fold increase in the apo-B48/B100 ratio has been reported (7). Many questions remain unanswered concerning the influence of the hormonal and nutritional disorders in the obese Zucker rats on the molecular regulation of apolipoprotein synthesis in the liver and in the intestine. Studies of lipoprotein metabolism in vivo or in perfused liver make difficult the estimation of the net synthetic capacity of the organ since de novo synthesis, secretion and catabolism take place simultaneously (8). In order to obtain further information on the relative synthesis rate of apolipoproteins in the liver and the intestine from obese and lean Zucker rats, mRNA from the two organs were extracted and translated in vitro and the apolipoproteins A-IV and C were immunoprecipitated and electrophoresed. Our results support the view that overproduction of lipoproteins by the liver is the main factor in the development of Zucker's rats hyperlipemia and they also suggest differences in the transcriptional regulation of apolipoprotein synthesis in the liver and the intestine.

MATERIALS AND METHODS

Materials and animals : Guanidine hydrochloride (Gdn-HCl) and cesium chloride were purchased from Bethesda Research Laboratories, dithiothreitol and ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma Chemicals Co (St Louis Md). ^{35}S-methionine (1330 Ci/mmole) were purchased from the CEA (Saclay- France). Lean heterozygous (Fa/-) and obese homozygous (fa/fa) female Zucker rats were obtained from CSEAL-CNRS (Orleans-la-Source France) and used at the age of 7 - 9 weeks.

Preparation of RNA from rat hepatocytes and enterocytes : Rats were anesthetized with diethylether. Hepatocytes were isolated from rat liver after dissociation of the tissue with EDTA (9) ; jejunal and ileal segments of the small intestine were quickly removed and flushed with 0.13 M NaCl solution containing 100 µg heparin/ml at 4°C. ; mucosal cells were isolated using the technique of Weiser (10). Total RNA was extracted from hepatocytes and enterocytes using the guanidine-HCl method (11). In order to avoid ribonucleases activity, all glassware and aqueous solutions used in the preparation were treated with 0.1 % (v/v), diethyl pyrocarbonate and autoclaved at 120° C for 20 min.

Isolation of lipoproteins and apolipoproteins : The rats were anesthetized with ether. Their blood was sampled from the abdominal aorta into plastic tubes containing 1 mg/ml of di sodium EDTA and centrifuged at 2 000 g for 20 minutes. After addition of sodium azide, the lipoproteins were isolated by sequential ultracentrifugation in a Spinco L50 ultracentrifuge (Beckman, USA) at the following densities : VLDL d < 1.006, LDL d < 1.063, HDL d < 1.210. Apo-A-IV and apo-C were prepared after VLDL and HDL delipidation by column chromatography (12,13,14).

Preparation of antibodies : Antisera against rat apo-A-IV and apo-C were obtained by injecting rabbits in both hind foot pads with 1 mg of

purified apolipoprotein emulsified with 1 ml of Freund's adjuvant. (15) Antisera specificity was tested by immunodiffusion and immunoelectrophoresis.

Cell free translation system and immunoprecipitation : RNA was translated in a protein-synthesizing system from rabbit reticulocyte lysate (16). Inactivated staphylococcus aureus (IgG Sorb, the Enzyme Center, Boston, MA) were extensively washed and prepared according to the method of Kessler (17), and immunoprecipitation of the translation medium was done as described previously (18).

Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) Total translation products and immunoprecipitated proteins were solubilized in SDS and electrophoresed on 8 % or 15 % polyacrylamide slab-gels using the Laemmli buffer system (19). Autoradiography of dried slab gels was performed using Kodak X-omat films. Standard proteins of known molecular weight, as well as native apo-A-IV and apo-C from rat plasma HDL and VLDL, were run on the same gel and the relative positions of radioactive bands were compared to those of reference proteins.

RESULTS AND DISCUSSION

Many published reports on fa/fa and Fa/- Zucker rats show that the obese homozygous presents with an hyperlipemia, a liver steatosis with triglyceride accumulation (2). The intestinal mucosa, however has a normal lipid content (Table 1) in both phenotypes.

RNA was isolated, as described, from hepatocytes and enterocytes of lean and obese Zucker rat, and further translated in the presence of ^{35}S-methionine in a mRNA-dependent protein synthesizing system derived from rabbit reticulocyte lysates. Preliminary experiments were carried out in order to optimize the system. Maximal protein synthesis was obtained by addition into the assay of 10 µg hepatic RNA or 12.5 µg of intestinal RNA. Under optimal conditions, using equivalent amounts of liver RNA from lean and obese rats, we could notice the same efficiency in ^{35}S-methionine incorporation into protein in the two assays.

Electrophoretic analysis of the total translation products on SDS-polyacrylamide gels showed that hepatocytes mRNA directed the synthesis of many proteins with molecular weights ranging from 8000 to

TABLE 1 . Lipid composition of intestinal cells from lean and obese female Zucker rats

		LEAN RATS	OBESE RATS
TRIGLYCERIDE	nmoles/mg protein	29.19 ± 3.4	28.5 ± 2.8
CHOLESTEROL	nmoles/mg protein	114.00 ± 10.0	103.0 ± 9.7
PHOSPHOLIPID	nmoles/mg protein	208.00 ± 18.0	212.0 ± 20.0

Values represent the mean ± S.E.M. (n = 5)

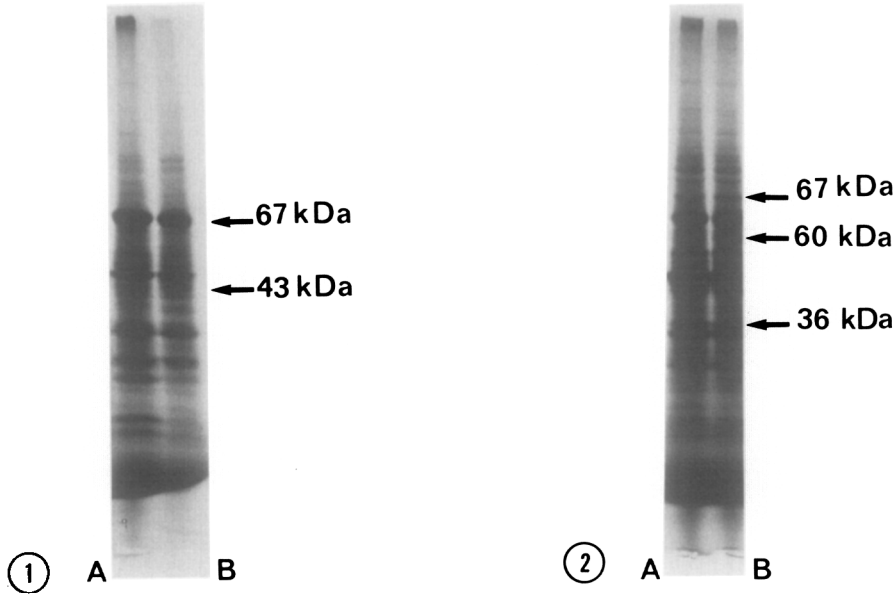
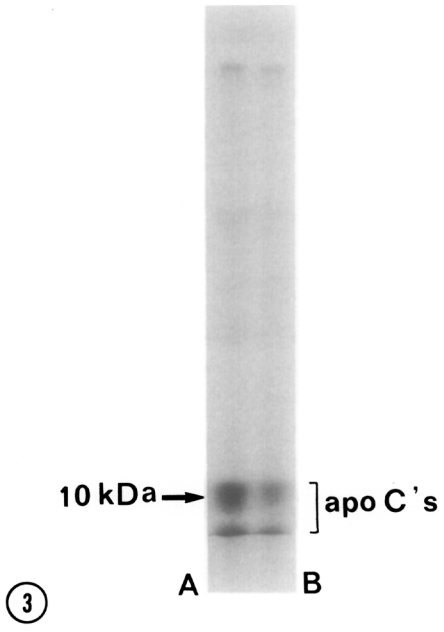


Figure 1 . Autoradiography of PAGE slab of translation products of liver mRNA from hyperlipemic (fa/fa) (A) and normolipemic (Fa/-) (B) Zucker rats.

Figure 2 . Autoradiography of PAGE slab of translation products of intestinal mRNA from fa/fa (A) and Fa/- (B) Zucker rats.

95000 daltons with differences in intensity of some bands between the RNA preparation of hepatocytes of lean and obese rats (Fig. 1). Autoradiography of PAGE slabs of translation products, of intestinal mRNA from lean and obese Zucker rats, did not show apparent differences between the protein bands synthesized in vitro (Fig. 2).

After immunoprecipitation of the labelled polypeptides with total apo-C antiserum, autoradiography of PAGE slabs revealed two bands, with an apparent molecular weight of approximately 8000 daltons and 10000 daltons respectively (Fig.3). Comparison of the immunoprecipitated proteins translated from mRNA extracted from identical amounts of hepatocytes from lean and obese rats shows a higher synthesis rate of apo-C's and indirectly a higher content of the corresponding mRNA in the hepatocytes of obese Zucker rats. On the contrary, this difference was not found after translation of intestinal RNA from obese and lean rats (Fig.4). The C-apolipoproteins are associated in the plasma with both the triglyceride-rich lipoproteins (VLDL, chylomicrons) and the HDL (20,21). The most abundant C-apolipoprotein is apo-C-III with an estimated Mr of 10000. In the rat apo-C-III is synthesized in the liver and to a lesser extent in the intestine (22). In vivo, after administration of a fat meal an increase in translatable apo-C-III mRNA was observed (23). Apo-C-I and



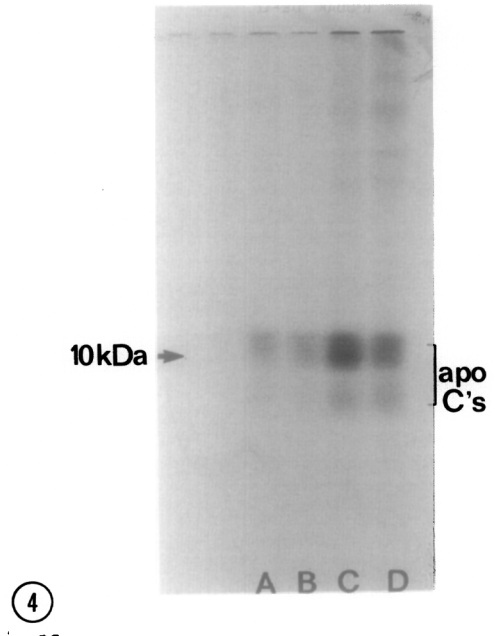


Figure 3 . Autoradiography of ^{35}S-methionine labelled apo-C's synthesized in a cell free system by hepatic mRNA from (fa/fa) (A) and Fa/- (B) Zucker rats. Electrophoresis was performed on 15 % polyacrylamide slab gel.

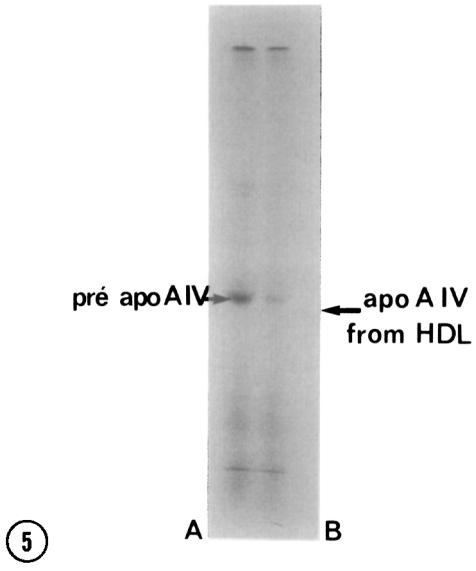
Figure 4 . Autoradiography of ^{35}S-methionine labelled apo-C's synthesized in a cell free system by intestinal mRNA from fa/fa (A) and Fa/- (B) and liver mRNA from fa/fa (C) and Fa/- (D) Zucker rats. Electrophoresis was performed on 15 % polyacrylamide slab gel.

apo-C-II are also constituents of VLDL and HDL. Patients with inherited apo-C-II deficiency, develops severe hypertriglyceridemia and lack functional activation of lipoprotein lipase (24).

Translation in a cell free system of hepatic mRNA from fa/fa and Fa/- Zucker rats (Fig. 3) revealed two bands corresponding probably to apo-C-III and to apo-C-II. The relative intensity of these bands is higher in preparations from obese rats. Our results on hepatic apo-C synthesis are in agreement with previous reports showing an increased amount of apo-C in plasma of obese rats compared to lean littermates (3).

Contrasting with the metabolic adaptation of the liver, the apo-C synthesis in intestinal cells of obese rats, is not increased (Fig. 4, A-B), in spite of the higher triacylglycerol synthesis rate and triglyceride-rich lipoprotein production.

For comparative purposes we have calculated the relative abundance of apo-C's mRNA in hepatocytes and enterocytes isolated from lean and obese rats. To do this, the incorporation of ^{35}S-methionine into immunoprecipitable proteins was expressed as a percentage of total protein synthesis in the assay. Apo-C mRNA represents 0.5 % of the total



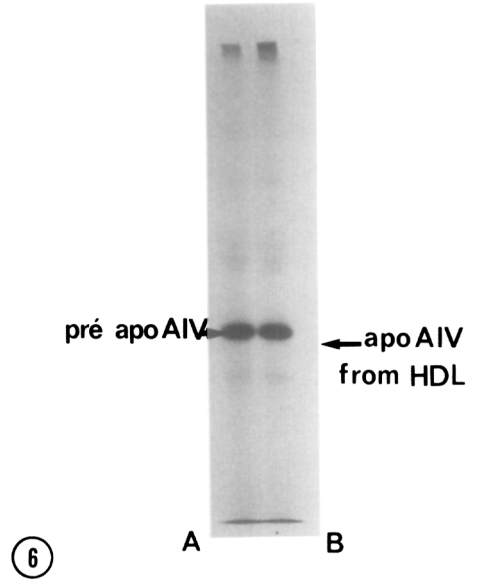


Figure 5 . Autoradiography of ^{35}S-methionine labelled pre-apo-A-IV synthesized in a cell free system by hepatic mRNA from fa/fa (A) and Fa/- (B) Zucker rats. Electrophoresis was performed on 8 % polyacrylamide slab gel.

Figure 6 . Autoradiography of ^{35}S-methionine labelled pre-apo-A-IV synthesized in a cell free system by intestinal mRNA from fa/fa (A) and Fa/- (B) Zucker rats. Electrophoresis was performed on 8 % polyacrylamide slab gel.

translatable mRNA in liver of lean rat and 0.9 % in the obese rat. In intestine of lean and obese rat, apo-C mRNA accounts for 0.15 % of the total translatable mRNA in our experimental conditions.

For a better understanding of the role played by intestinal lipoprotein secretion in hyperlipemia of obese rats, we have also studied the synthesis of apo-A-IV by RNA isolated from hepatocytes and enterocytes of lean and obese Zucker rat. Indeed, apo-A-IV is synthesized by the intestine and also by the liver (25).

Apo-A IV mRNA levels in intestine is about four times higher than in liver cells preparations. Some studies have shown that four hours after feeding corn oil, rat intestinal apo-A-IV mRNA increases (18,26). Immunoprecipitation of the labelled polypeptides with apo-A-IV antiserum revealed a single protein band with an apparent molecular weight of 48000 daltons, 2000 daltons larger than mature plasma apo-A-IV. This finding is in agreement with previous reports (27) showing that the primary translation products of apo-A-IV's mRNA is a pre-protein containing a 20 aminoacid N terminal extension.

Comparison of the immunoprecipitated proteins from obese and lean Zucker rats show an increased capacity of apo-A-IV synthesis in

hepatocytes from fa/fa rats (Fig. 5); conversely no difference was noted between the synthesis rate of intestinal cells of lean and obese rat (Fig. 6). The relative abundance of apo-A-IV mRNA has been calculated. Rat hepatocyte apo-A-IV mRNA increase from 0.2 to 0.4 % of total mRNA in obese rats ; whereas apo-A-IV mRNA levels from intestine of lean and obese rats are not affected (0.75 %).

In conclusion, we have shown that the liver cells from obese Zucker rats contain a higher level of mRNA coding for apo-C and apo-A-IV and express a higher synthetic rate for these apolipoproteins compared with lean rat liver. Presumably, this difference represents an adaptation to increased hepatic triglyceride synthesis in fa/fa rats. A similar increase has not been noticed in the intestinal cells in spite of the fact that in the obese hyperphagic rat, enterocytes are under continuous stimulation by triglyceride synthesis and chylomicron production. These results suggest that the hyperlipoproteinemia of homozygous (fa/fa) Zucker rat is mainly or exclusively due to an overproduction of lipoproteins by the liver.

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