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Hormonal and Nutritional Stimuli Modulate Apolipoprotein B mRNA Editing in Mouse Liver

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SUMMARY Human livers produce apoB-100, a major protein of VLDL, while intestines produce apoB-48, the major protein of chylomicrons. ApoB-48 is translated from apoB-100 mRNAs that are post-transcriptionally edited at codon 2153, converting CAA (glutamine) to TAA, a stop codon. In contrast to humans, mouse and rat livers contain the apoB-100 mRNA editing mechanism. Because hormones and nutrients affect the metabolism of apoB containing lipoproteins, we studied the effects of sex hormones and diets on apoB mRNA editing. Groups of male and female C3H/HeJ mice were castrated and treated with 17 ß-estradiol at 0.16 (E2L) or at 5 μg (E2H), or with testosterone propionate at 1 μg/g body weight/day for 14 days. Plasma apoB levels and ratios of apoB-100/apoB-48 both increased 2-fold, but only in the E2H group. To determine if the increased apoB-100/apoB-48 ratios were associated with altered levels of apoB-100 and apoB-48 mRNA, both forms of apoB mRNA were quantified. We found that indeed ApoB-100 mRNA increased 1.8-fold ($p<0.025$) compared to apoB-48 mRNA only in the E2H group. Next, we studied the individual effects of dietary fatty acids and dietary cholesterol on the relative abundance of apoB-100 and apoB-48 mRNA. Contrary to the estrogen effect, the high fat-combination diet increased apoB-48 mRNA relative to apoB-100 mRNA. Total plasma apoB as well as apoB-48 synthesis in liver also increased. Our studies demonstrate that estrogens and high fat diet both modulate apoB editing in mouse liver, but that estrogens and fat diet affected apoB mRNA editing in opposite directions.

Plasma apolipoprotein B exists in two molecular forms, apoB-100 and apoB-48 (1). ApoB-100 is the full length apoB protein while apoB-48 is a smaller protein accounting for amino terminal 48% of the full length of the apoB molecule. In humans, apoB-100 is synthesized by the liver and secreted as a structural component of VLDL. ApoB-48 is synthesized by the intestine and is an obligate component of triglyceride-rich chylomicrons (2, 3). By contrast, rat and mouse livers synthesize both apoB-100 and apoB-48 (4). Thus, rodent livers are suitable organs in which to study the production of apoB-100 and apoB-48. The formation of the shorter form of apoB results from editing of C→T at codon 2153 (5, 6). This editing mechanism converts CAA (glutamine) to TAA (an in-frame translational stop codon) giving

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rise to apoB-48 mRNA. The editing activity that introduces a T in place of C at codon 2153 has been partially characterized (6-8). Thyroid hormone administration and high carbohydrate diets increase apoB editing in rats (9,10). ApoB mRNA editing is also developmentally regulated (11). We have observed that pharmacological doses of estradiol increase plasma apoB levels and LDL concentrations in mice (12). Since apoB-100 is the main protein constituent of LDL, we hypothesized that following estrogen administration hepatic apoB-100 production should increase along with concomitant increases in hepatic apoB-100 mRNA levels. We also have found that plasma apoB levels increase in fat-fed mice. In order to assess which form of apoB was affected by these perturbations, we quantified apoB-100 and apoB-48 mRNA in livers of mice.

MATERIALS AND METHODS

Animals and Treatment Groups

Male and female C3H/HeJ mice were obtained from Jackson Laboratory, Bar Harbor, ME., at ages of 5-6 weeks and were fed Purina Chow 5015 and tap water *ad libitum*. Each gender was divided into four groups (n=5, in each group). Group 1: Placebo, castrated mice treated with the propylene glycol vehicle; Group 2: Castrated mice administered 0.16 μg 17 ß-estradiol/g body weight/day (E2L); Group 3: Castrated mice administered 5 μg 17 ß-estradiol/g body weight/day; and Group 4: Castrated mice administered 1 μg testosterone proportionate/g body weight/day. Animals were allowed to recover for 14 days after surgery. Hormones were administered subcutaneously for 14 days at the same time each day. Then, mice were fasted for 16-18 hours and killed by exsanguination. Plasmas from individual mice anticoagulated with 1 mM EDTA were pooled by treatment group and gender for apolipoprotein determination. Livers were removed immediately for the preparation of total RNA (13).

For the diet studies, groups (n=4) of male C3H/HeJ mice were fed control diet (5% corn oil), control plus 0.5% cholesterol (high cholesterol diet) and control plus 20% hydrogenated coconut oil (high fat diet) for two weeks.
At the end of the experiment, mice were fasted overnight and sacrificed for plasma apolipoprotein analysis. Total RNA was prepared immediately after excising the livers (13).

Determination of Plasma ApoB Levels and hepatic apoB synthesis

Total plasma apoB levels were quantified by electroimmunoassay as described (14). In some cases apoB-100 and apoB-48 levels were determined by fractionating the plasma apolipoproteins by gradient SDS-gel electrophoresis and scanning the apoB-100 and apoB-48 bands in Coomassie blue stained gel. Determination of apoB-100 and apoB-48 syntheses were performed by incubating ^{35}S-methionine with pooled liver slices, followed by homogenization of tissues, immunoprecipitation of apoB and SDS-gel electrophoretic separation of apoB-100 from apoB-48 (15). The bands for apoB-100 and apoB-48 were visualized by autoradiography. Bands were scanned and relative levels of apoB-100 and apoB-48 were determined.

Quantification of ApoB-100 and ApoB-48 mRNA

ApoB-100 and apoB-48 mRNAs were quantified in livers by reverse transcriptase-polymerase chain reaction coupled to primer extension assay as described (9, 10, 16). For apoB editing assay, the region of mRNA flanking the edited site was reverse transcribed and amplified by PCR. Five μg total liver RNA were dried in a speed-vac and redissolved in 62

μl water. PCR was performed in 100 μl assay volume in the presence of 200 μM of all the four dNTPs, 1X PCR buffer, 2 mM $MgCl_2$ and 1 μg of each of the primers, 5'ATC TGA CTG GGA GAG ACA AGT AG3' AND 5' CAG GGA TAT GAT ACT GTT CGT CAA GC3'. The PCR kit was obtained from Perkin Elmer Cetus, CT. The contents were heated at 70°C for 10 min and 4 units of AMV reverse transcriptase were added and reaction performed at 50°C for 10 min. The reaction mixture was incubated at 91°C for 2 min to inactivate reverse transcriptase followed by the addition of 2.5 units of *Taq* DNA polymerase. Thirty cycles of PCR were performed using the following profiles: 92°C/1 min; 58°C/6 min. An aliquot of the PCR product was checked on a 2% agarose gel for the correct size of the PCR product. The amplified DNA was purified on a Sephadex G-25 column and used for the editing assay. Two hundred nanogram of amplified DNA were used for each editing assay using the end ^{32}P labeled primer 5'ATA ATA ATT ATC TCT AAT ATA CTG A3' (16).

RESULTS

Effects of Estradiol Administration on ApoB mRNA Editing

We have earlier shown that administration of physiological doses (0.16 μg/g body weight/day, E2L group) of estradiol to castrated mice did not affect either plasma LDL or apoB concentrations in plasma (12). However, administration of pharmacological doses of estradiol (5 μg/g body weight/day, E2H group) increased plasma LDL as well as apoB concentrations by about 2-fold (12). Concomitantly, hepatic apoB mRNA levels also increased in the E2H group (12). We now report that plasma apoB-100/apoB-48 ratios also increased but only in the E2H group, by about 2-fold in the male and about 1.6-fold in the female mice (Table 1). Similarly, the ratios of hepatic apoB-100/apoB-48 mRNA increased

Table 1

Effects of estradiol and testosterone administration on plasma apoB-100/apoB-48 and hepatic apoB-100/apoB-48 mRNA ratios

Treatment Groups	Plasma apoB-100/B-48[a]		Hepatic apoB-100/B-48 mRNA[b]	
	Female	Male	Female	Male
Placebo	100	100	100±8	100±11
E2L	109	116	79±13	67±17
E2H	164	202	*126±12	*198±9
Tp	93	84	88±10	104±9

[a]Two and 4 μl of delipidated plasma were separated in 3%/6% polyacrylamide SDS gel. Plasma samples were run in duplicate. ApoB-100 and B-48 protein were stained with Coomassie Blue and the intensity of the bands was analyzed by the image analysis system. Each value in the table represents an average of two estimations and is expressed as the dye area ratio x 100. Placebo group mean area ratio has been asigned a value of 100%.
[b]Ratios of apoB-100/B-48 mRNA were determined as shown in Figure 1. The mean apoB-100/B-48 mRNA area x 100 for the placebo group has been asigned a value of 100% and rest of the values are relative to the placebo group. E2L, 0.16 μg/g body weight/day estradiol; E2H, 5 μg/g body weight/day estradiol; Tp, 1 μg/g body weight/day testosterone.
* significantly different compared to placebo group ($p<0.025$).

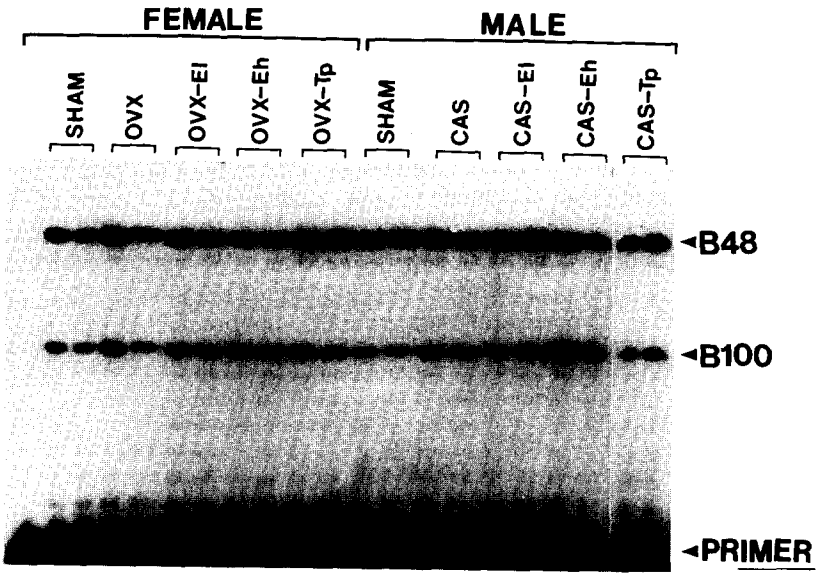

Figure 1. ApoB mRNA editing assay. Five microgram total liver RNA was reverse transcribed and amplified by PCR using appropriate primers as described in the materials and methods section. Two hundred nanograms of amplified DNA was used for the apoB mRNA editing assay. The left most lane shows primer alone and the rest of the lanes have been labeled. Sham, sham operated; Ovx, ovarectomized; Ovx-El, ovarectomized and 0.16 μg estradiol administered; Ovx-Eh, Ovarectomized and 5 μg estradiol administered; Ovx-Tp, ovarectomized and 1 μg testosterone administered; Cas, castrated. After the editing assay, the extension products were separated in a sequencing gel. The positions of apoB-100 mRNA-specific and apoB-48 mRNA-specific bands are marked in the figure.

significantly both in male and female mice (Figure 1, Table 1). Increases in the ratios of apoB-100/apoB-48 mRNA were more pronounced in the male (2-fold) compared to the female (1.3-fold) mice. Testosterone administration did not alter plasma LDL concentrations, plasma apoB-100/apo-48 ratios or hepatic apoB-100/apoB-48 mRNA ratios, and this was true for both genders (Figure 1 and Table 1).

Effects of High Cholesterol and High Fat Diets on ApoB mRNA Editing

We have observed previously that plasma apoB concentrations increase on high fat diet but not on the high cholesterol diet (14). We now report that the high cholesterol diet did not affect the apoB mRNA ratios while high fat diet increased apoB-48 mRNA relative to apoB-100 mRNA (Figure 2, Table 2). The synthesis of apoB-48 increased relative to apoB-100 in liver slices of fat-fed mice compared to control or cholesterol-fed mice (Table 2). The combination diet mimicked the high fat diet with respect to both the protein and mRNA ratios.

DISCUSSION

The aim of the present investigation was to assess the effects of sex hormones and two dietary factors, cholesterol and saturated fatty acids, on apoB mRNA editing in livers

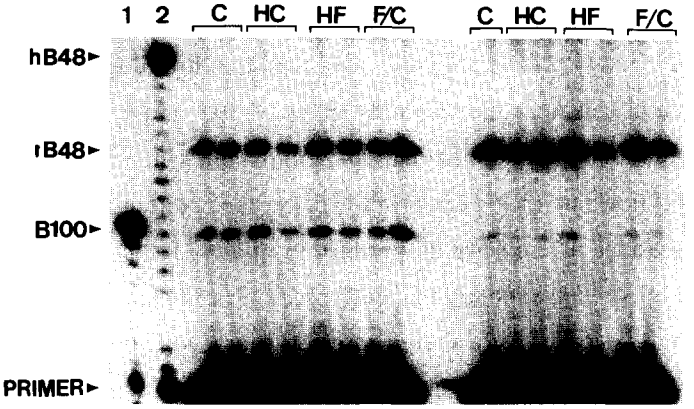

Figure 2. Effects of feeding high cholesterol and high fat diet on apoB mRNA editing. Groups of male C3H mice were fed control diet (C, 5% corn oil); high cholesterol diet (HC, 0.5% cholesterol); high fat diet (HF, 20% coconut oil) or combination of HC and HF (F/C). ApoB mRNA editing assay was performed with liver RNA isolated from mice of each group. Lanes 1 and 2 represent control assay with synthetic 59 mer DNA based on human apoB gene sequence flanking the edited site, one containing C (lane 1) at nucleotide 6666 and the other containing T (lane 2) at nucleotide 6666. hB48, human B48; rB48, rodent B48. Rodents apoB gene has a C at nucleotide 6661 so that the reaction terminates at first C giving rise to shorter extension product compared to human which does not have a C at nucleotide 6661. However, both human and rodents have a C at nucleotide 6655. The left panel represents liver and the right panel intestine.

of C3H/J mice. We have shown previously that some of these perturbations can affect plasma apoB concentrations and hepatic apoB mRNA levels in these animals (12,14). Others have shown that thyroid hormone (9) and high carbohydrate diets (10,17) also affect apoB-100/apoB-48 plasma ratios and apoB mRNA editing. Therefore, we hypothesized that sex hormones and dietary fat and cholesterol too could differentially affect hepatic apoB-100 and apoB-48 production. To test this hypothesis, we assessed apoB-100/apoB-48 ratios in plasma,

Table 2

Effects of feeding high cholesterol and high fat diets on hepatic apoB-100/B-48 mRNA and apoB-100/B-48 synthesis ratios

Diet Groups	Hepatic apoB-100/B-48 mRNA ratios	Hepatic apoB-100/B-48 synthesis ratios
Control	100±8	100±12
High Cholesterol	86±22	96±17
High Fat	45±18*	70±11*
High Fat/Chol	58±18*	70±30

Hepatic apoB-100/B-48 mRNA ratios were determined by apoB editing assay as shown in figure 2. See also Table-1. * significantly different compared to placebo group ($p<0.05$).

or relative rates of synthesis of the two forms of apoB by liver slices and the relative hepatic level and apoB-100 and apoB-48 mRNAs. We found that high doses of estrogen indeed do selectively increase plasma concentrations of apoB-100 and relative hepatic levels of unedited apoB-100 mRNA. These findings are compatible with a selective enhancement of the overall production rate of hepatic apoB-100, perhaps accounting for the selective rises of apoB100 in plasma. Altered overall production of apoB100 could be due to enhanced mRNA translation or secretion or the decreased intracellular degradation of apoB100 compared to apoB-48. Selectively altered rates of clearance from plasma also could raise apo-B100 levels. Formal kinetic studies need to be done to settle the issue. However, it is unlikely that enhanced level in plasma apoB-100 was due to estradiol effect on selective clearance of apoB100 because the high doses of estrogen administrations enhanced hepatic LDL-receptor expression in many animals which should lead to lower plasma concentrations of apoB-100. Although the mouse is uniquely resistant to the LDL-receptor enhancing activity of estrogen, LDL-receptor expression is certainly not suppressed. Therefore, our tentative conclusion remains that estrogen-induced enhanced plasma apoB-100 levels in mouse are a consequence of down regulation of apoB-mRNA editing leading to increased rates of production of apoB-100. This, combined with increases of hepatic triglyceride production (18) leads to increased rates of hepatic VLDL assembly and secretion and rises in plasma apoB-100.

Dietary cholesterol did not affect apoB-mRNA editing but dietary fatty acids had effects opposite to those produced by estradiol. Extent of ApoB-48 mRNA editing increased and apoB-48 concentrations in plasma increased relative to apoB-100. Again, intracellular and secretory events and plasma kinetics of apoB remains to be studied, but using the above chain of reasoning our tentative conclusions are that the fatty acids induced enhancement of hepatic apoB-100 mRNA editing and fatty acids induced rises in plasma apoB-48 are directly related to each other. The contribution of the intestine to plasma apoB-48 concentrations under these conditions also remains to be determined.

It is not clear whether estrogen and fatty acids affect the extent of apoB mRNA editing by altering the activity of the intranuclear editing complex (17,19). Other possibilities could be altered transport of mRNA out of the nuclei thereby altering the intranuclear apoB100 "substrate" levels of the editing complex. Altered rates of cytoplasmic degradation of the edited or the unedited apoB mRNA also could occur. Finally, it is worth noting that both of these perturbations, estrogens and dietary saturated fatty acids, probably stimulate the overall secretion of VLDL triglyceride and VLDL-apoB, yet the form of apoB that is accompanied the secreted VLDL differs for the two perturbations. Clearly much remains to be learned about assembly and secretion of VLDL.

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