PRELIMINARY REPORT

Regulation of Hepatic Apolipoprotein B RNA Editing in the Genetically Obese Zucker Rat

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Hepatic apolipoprotein (apo) B RNA editing was examined in the genetically obese hyperinsulinemic and hypertriglyceridemic Zucker rat. In obese Zucker rats, apo B RNA editing was increased 42% relative to that in lean controls. Correspondingly, the proportion of serum triglyceride-rich lipoprotein containing apo B48 increased 4.7-fold in the obese Zucker rat. Quantification of hepatic total apo B mRNA showed no difference between obese Zucker and lean control rats. In contrast, the hepatic mRNA encoding APOBEC-1, the catalytic subunit of the RNA editing activity, demonstrated an increased abundance of 1.8-fold in obese Zucker rats versus lean controls.

APOLIPOPROTEIN (apo) B RNA editing involves the deamination of cytidine to form uridine at nucleotide position 6666, and results in the translation of the truncated apo B48 protein due to the formation of a premature translation stop codon.[1,2] Regulation of apo B RNA editing has been shown to be species- and tissue-specific, and occurs during development and following metabolic perturbation.[2]

The catalytic subunit (APOBEC-1) of the editing complex has been identified.[2] Experimentally induced overexpression of *apobec-1* mRNA results in increased apo B RNA editing activity.[1,2]

Insulin has a major effect on hepatic and serum triglyceride-rich lipoprotein profiles,[3] and long-term administration of insulin to primary hepatocytes increases apo B RNA editing efficiency and apo B48 secretion.[4] The genetically obese (*fa/fa*) Zucker rat has been used extensively as an animal model system for chronic hyperinsulinemia, insulin resistance, and hypertriglyceridemia in studies of lipoprotein metabolism.[5] We evaluated herein whether the efficiency of hepatic apo B RNA editing is altered in this animal system.

MATERIALS AND METHODS

Male obese Zucker and lean control rats were obtained from Charles River Laboratories (Wilmington, MA). Rats were maintained on normal rat chow under controlled lighting conditions. Blood profiles on 10- and 20-week-old obese Zucker rats demonstrated that they were characteristically hyperinsulinemic and hypertriglyceridemic.[5]

Total liver RNA was isolated as described previously,[1-3] and 10 μg from each tissue was resolved through 1% agarose gels and transferred to nylon membranes. Alternatively, 2.5 to 25 μg total hepatic RNA was prepared as dot blots. Filters were prehybridized overnight at 42°C in 50% formamide, 0.75 mol/L NaCl, 50 mmol/L NaH_2PO_4, 5 mmol/L EDTA, pH 7.4, 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 0.1% Ficoll 400, 1% sodium dodecyl sulfate, and 100 μg/mL salmon sperm DNA. Blots were hybridized with either [α-^{32}P]dATP random hexamer–labeled *apobec-1* cDNA, β_2-microglobulin cDNA,[6] or an 18-mer of oligo(dT) (total poly(A)$^+$ mRNA probe). The blots were washed four times at 50°C to a final stringency of 0.5× SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate), and stripped between each hybridization in 2× SSC at 100°C for 30 to 60 minutes.

The editing efficiency of apo B RNA was determined on products from the reverse transcriptase–polymerase chain reaction using rat apo B mRNA–specific primer pairs.[1] The edited base was quantified on reverse transcriptase–polymerase chain reaction products by the poisoned primer extension analysis described previously.[1] Editing efficiency was calculated as the amount of primer extension product corresponding to edited apo B RNA (UAA) divided by the sum of unedited (CAA) and UAA RNAs times 100.

Northern blots and editing assays were quantified by laser scanning densitometry (PhosphorImager Model 425E; Molecular Dynamics, Sunnyvale, CA).

Serum lipoproteins were subjected to electrophoresis and evaluated by immunoblotting with apo B–specific monoclonal antibodies as described previously.[7]

RESULTS AND DISCUSSION

We have previously demonstrated that streptozotocin-diabetic and normal rats have similar levels of hepatic apo B RNA editing efficiency.[3] However, long-term insulin treatment of normal primary rat hepatocytes increased the editing of apo B RNA and secretion of apo B48–containing lipoproteins.[4] Earlier studies have shown that the ratio of apo B48/apo B100 in very–low-density lipoproteins (VLDL) from obese Zucker rat liver perfusates was three times higher than that obtained from lean controls.[8] We have quantified the ratio of apo B48/apo B100 in serum VLDL of obese Zucker and lean control rats by Western blotting using a monoclonal antibody equally reactive with the two proteins.[7] The ratio of apo B48/apo B100 in serum VLDL from 10- and 20-week-old obese Zucker rats was

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(mean ± SD) 2.7 ± 0.8 (n = 4) and 5.6 ± 1.2 (n = 3), compared with 1.2 ± 0.2 (n = 4) in lean controls. The abundance of hepatic total apo B mRNA was quantified on dot blots by probing with radiolabeled apo B cDNA and oligo(dT) (total poly(A)$^+$ mRNA). The signal ratio for apo B to total poly(A)$^+$ mRNA was 0.30, 0.28, and 0.27 for lean controls and 10- and 20-week-old obese Zucker rats (n = 2), respectively, suggesting that there was no significant difference in apo B mRNA abundance.

Hepatic apo B RNA editing efficiency of lean rats was 47.5% ± 7.8% (n = 3), and was significantly lower than that observed in livers of both 10- and 20-week-old obese Zucker rats (61% ± 6.2%, n = 4, and 67% ± 2.2%, n = 3, respectively; Fig 1). Editing efficiency in the small intestine averaged 90% in both obese and lean Zucker rats (data not shown).

The low abundance of hepatic APOBEC-1 protein has made direct quantification of it difficult. Recent studies have shown that elevated levels of hepatic *apobec-1* mRNA directly correlated with elevated levels of apo B RNA editing and circulating levels of apo B48–containing lipoproteins.[2] We evaluated this correlation by quantification of Northern blot hybridization for mRNAs encoding *apobec-1* and β_2-microglobulin. The abundance of the latter has been demonstrated to be largely refractory to metabolic and developmental regulation.[6] The signal ratio of *apobec-1* to β_2-microglobulin mRNAs in either 10- or 20-week-old obese Zucker rats was 1.8-fold the ratio in RNA from lean controls (Fig 2).

The data presented herein demonstrate changes in hepatic apo B RNA editing efficiency in hyperinsulinemic obese rats, and suggest that increased expression and/or stability of *apobec-1* mRNA is directly responsible for this effect and for the increased ratio of apo B48/apo B100 in serum VLDL of the obese Zucker rat. The potential applications of the genetically obese Zucker rat to the study of factors involved in apo B RNA editing will be of future interest.

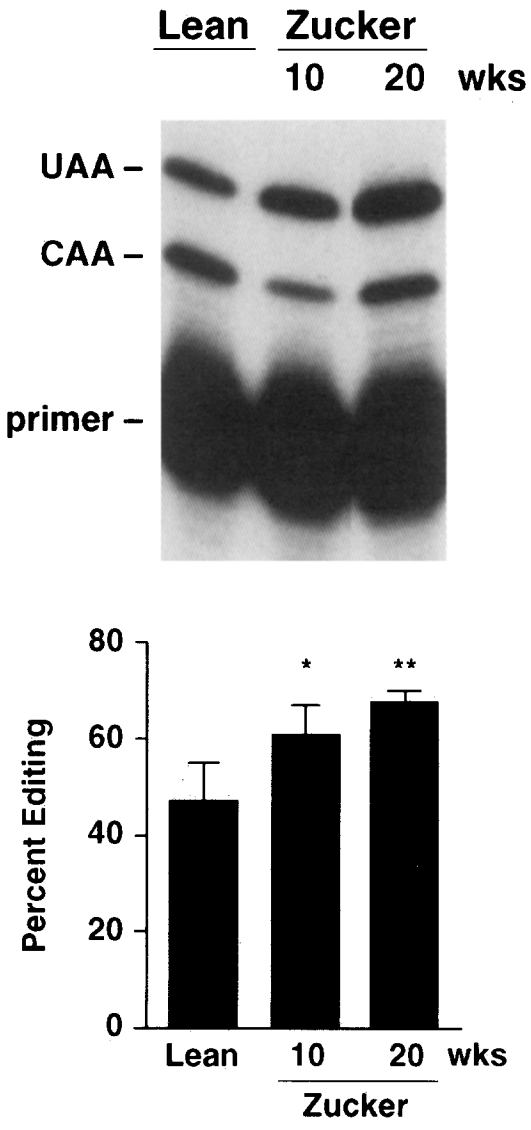

Fig 1. RNA editing primer extension assay (top) and its quantification (bottom). *P = .037, **P = .008.

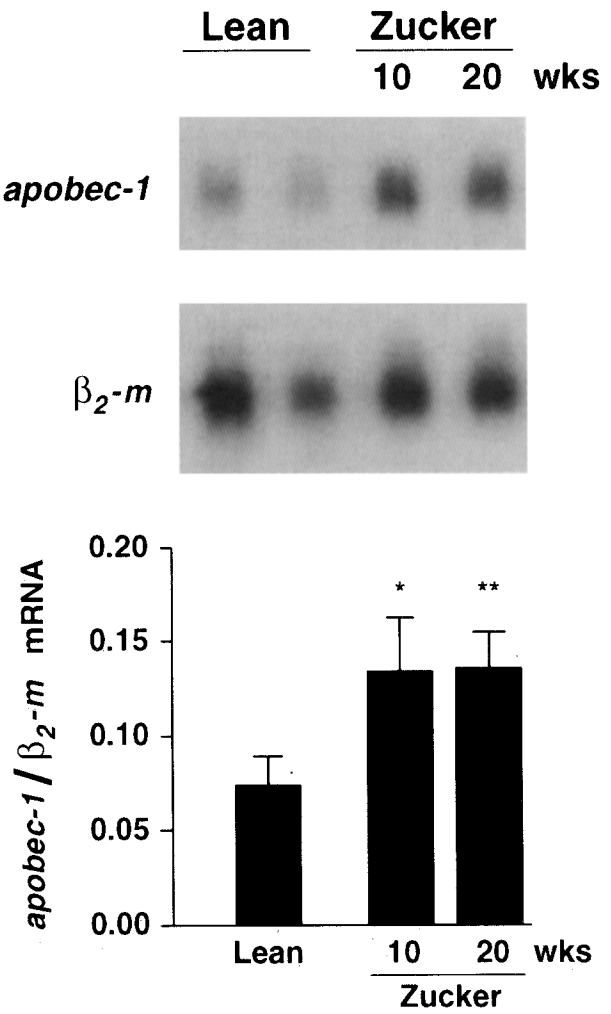

Fig 2. Northern blot hybridization (top) and its quantification (bottom). *P = .015, **P = .005.

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