# Insulin Increases Expression of apobec-1, the Catalytic Subunit of the Apolipoprotein B mRNA Editing Complex in Rat Hepatocytes

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**We have previously shown that chronic insulin treatment of rat hepatocytes increases the fraction of edited apolipoprotein B (apoB) mRNA from approximately 50% to as much as 90%. We have now examined the effect of insulin on apobec-1 mRNA abundance and demonstrate that increased editing of apoB mRNA following insulin treatment is accompanied by elevated apobec-1 mRNA levels in primary rat hepatocytes. Time-course measurements of the effects of insulin on apoB mRNA editing and apobec-1 mRNA abundance showed that both were elevated almost maximally within 48 hours and sustained for at least 5 days of insulin treatment.**



APOLIPOPROTEIN B (apoB) is the main protein component of chylomicrons, very–low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL). An understanding of apoB synthesis is of critical importance, as increased levels of the apoB-containing lipoproteins in the plasma contribute to the development and progression of atherosclerosis.[2] ApoB is expressed in a full-length form, apoB100, and in a truncated form, apoB48, which is the amino-terminal 48% of apoB100. A common structural gene generates apoB100 and apoB48 through a novel posttranscriptional modification in which the CAA codon for glutamine-2153 in apoB is changed to UAA, resulting in an in-frame translation stop signal.[3] This process is mediated by a multicomponent complex containing a single catalytic subunit, apobec-1. Although the process occurs exclusively in the intestine in the human (the human liver secretes only full-length apoB100), other species such as the rat, mouse, horse, and dog also produce apoB48 in the liver.[4] The observation of low levels of LDL in these species, presumably due to the rapid clearance of apoB48-containing lipoproteins, has led to active interest in conferring this apoB mRNA editing capability to human liver as a potential means of reducing LDL.[5-8] Accordingly, the study of the regulation of hepatic apoB mRNA editing is important in fully understanding the ramifications of such therapy. Some factors than can modulate editing are known. Editing is reported to be increased by refeeding after a fast,[9] by thyroid hormone,[10] and by ethanol.[11] Cholesterol feeding, estradiol, and fasting, on the other hand, reduce apoB mRNA editing.[9,12,13]

We have previously shown that secretion of apoB48 is increased in primary rat hepatocytes cultured in the presence of insulin for 5 days.[14] This effect contrasts with the inhibition of apoB synthesis and secretion that is observed with acute insulin exposure.[15-17] At the same time, we observed an increase in the fraction of edited apoB mRNA.[14] A greater fraction of apoB mRNA in the edited form has also been observed in the livers of obese, hyperinsulinemic rats compared with normal lean controls.[18,19] Surprisingly however, insulin deprivation, as seen in streptozotocin-induced diabetes in rats, did not result in lower hepatic apoB mRNA editing,[20] suggesting that insulin may not be required for basal apoB mRNA editing. Quantitatively altered levels of edited versus unedited apoB mRNA in the hyperinsulinemic state implicate altered amounts and/or activities of the molecular components involved in apoB mRNA editing.

We have undertaken a study of the steady-state levels of apobec-1 mRNA using reverse transcriptase–polymerase chain reaction (RT-PCR), as well as RNase protection, after chronic insulin exposure of primary hepatocytes. We demonstrate that apobec-1 mRNA abundance is inducible, suggesting a mechanism for the insulin-stimulated enhancement of apoB mRNA editing.

## MATERIALS AND METHODS

### *Primary Hepatocyte Culture*

Adult male Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN) and fed ad libitum with rat chow (no. 5001; Ralston Purina, St Louis, MO). Preparation and culture of hepatocytes were as described,[21] with hepatocyte suspensions obtained using type 2 collagenase (Worthington, Freehold, NJ) and dispersion techniques as detailed previously,[21] adapted for monolayer culture according to Davis.[22] Bovine insulin (Sigma, St Louis, MO), at 400 ng/mL (67 nmol/L), was added to the feeding medium of half of the cultures. The medium, with or without insulin, was replaced daily. Radioimmunoassay (RIA) analysis using a kit from ICN (Costa Mesa, CA) indicated that the insulin concentration did not fall below 60 ng/mL before medium changes.

### *Preparation of RNA and cDNA*

After 5 days of culture in the presence or absence of insulin, total RNA was isolated from hepatocyte monolayers using RNA Stat-60 (Tel-Test "B," Friendswood, TX) according to the manufacturer's instructions and quantitated by absorbance at 260 nm. Following digestion with RNase-free DNase I (Ambion, Austin, TX) and heat denaturation of the enzyme, 5 μg of RNA from each pool of hepatocytes

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was used to prepare cDNA with the cDNA Cycle Kit (Invitrogen, San Diego, CA), using avian myeloblastosis reverse transcriptase.

### *PCR Amplification of apoB mRNA*

A 304 bp fragment of the rat apoB cDNA containing the edited site was amplified by RT-PCR using the cDNA samples generated from the hepatocyte RNAs as templates as described.[14]

### *Primer Extension Analysis*

Editing of apoB mRNA was quantitated by primer extension analysis using a 27-base oligonucleotide primer complementary to the region starting 8 bases downstream from the edited site in rat apoB mRNA as described by Wu et al.[23] The primer (10 pmol) was 5′-end-labeled using a kit from Promega (Madison, WI), using 150 μCi of [gamma-$^{32}$P]adenosine triphosphate (6,000 Ci/mmol; Dupont NEN, Boston, MA). Approximately 25 ng of the product from each PCR was assayed by denaturing the DNA at 95°C for 5 minutes, then annealing with a fivefold molar excess of labeled primer in 34 mmol/L Tris-HCl pH 8.3, 50 mmol/L NaCl, 5 mmol/L dithiothreitol at 58°C for 30 minutes. The extension was performed at 42°C for 30 minutes after the addition of dATP, dTTP, and dCTP to 0.5 mmol/L and 2′,3′-dideoxy-guanosine triphosphate to 3 mmol/L, plus 5 U of avian myeloblastosis virus reverse transcriptase (Invitrogen) according to Driscoll et al.[24] The reaction products were resolved on a 12% polyacrylamide sequencing gel and analyzed by autoradiography with Kodak X-Omat AR film (Eastman Kodak, Rochester, NY). The bands corresponding to edited or nonedited apoB mRNA were cut out of the gel and quantitated by liquid scintillation spectrometry.

### *RT-PCR of Apobec-1*

A 394-bp fragment from apobec-1 mRNA was amplified from the cDNA samples by PCR. Sample sizes and the number of amplification cycles were optimized to produce measurements within a linear range. Ethidium bromide–stained agarose gels of the reaction products were digitized using an Alpha Inotech (San Leandro, CA) image analysis workstation and the optical density of the DNA bands quantitated using National Institutes of Health (NIH, Bethesda, MD) Image software. Parallel independent amplifications of the cDNA for the ribosomal protein, L-32, or the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were performed in separate tubes to normalize sample sizes. These assays were also optimized to produce reactions in the linear range for L-32 and GAPDH. For apobec-1, the primers used were as follows: 5′-GAGAAGAATTGAGCCCCACGAG-3′ (nucleotides 86 to 107) and 5′-CTCCGTCATGATCTGGATAGTA-3′ (nucleotides 458 to 479). For L-32, the primers used were as follows: 5′-GTGAAGCCCAAGATCGTC-3′ (nucleotides 28 to 45) and 5′-GAACACAAAACAGGCACAC-3′ (nucleotides 422 to 440). The Amplimer Set for GAPDH from Clontech (Palo Alto, CA) was used to amplify a 452-bp fragment from the GAPDH cDNA. PCR reactions were performed using GeneAmp reagents from Perkin-Elmer (Foster City, CA) at the recommended concentrations. Conditions for apobec-1 and GAPDH PCR were 3 minutes at 94°C followed by 28 to 30 cycles of 50 seconds at 94°C, 2 minutes at 55°C, and 2 minutes at 72°C. At the end of the run, samples were incubated an additional 8 minutes at 72°C. Conditions for L-32 PCR were 2 minutes at 94°C followed by 27 cycles of 50 seconds at 94°C, 2 minutes at 50°C, and 2 minutes at 72°C, plus an additional 8 minutes at 72°C at the end of the run.

### *RNase Protection Assays*

A radiolabelled 339-nt antisense rat apobec-1 riboprobe (A 1,2,3 riboprobe)[25] was transcribed using T7 RNA polymerase (Promega, Madison, WI). Hybridizations were conducted with 50 μg total RNA under conditions detailed recently.[22] All reactions included a rat cyclophilin antisense riboprobe (Ambion), generated as a 165-nt fragment using T7 RNA polymerase. Following digestion with RNase A/T1 (Ambion), the mixtures were precipitated and resolved through 8% denaturing polyacrylamide gels. Autoradiographs were digitized using an Alpha Inotech image analysis workstation and the optical density of the protected apobec-1 and cyclophilin fragments quantified using NIH Image software.

## RESULTS

Data from three representative experiments, illustrating the insulin-mediated increase in apoB mRNA editing in primary rat hepatocytes, are shown in Fig 1. In five independent hepatocyte preparations, the percentage of edited apoB mRNA was increased following 5-day insulin treatment from an average of 55% to 78% (Table 1). These findings confirm and extend our previous observation that, in addition to increasing apoB48 synthesis and secretion, long-term insulin treatment enhances apoB mRNA editing.[14] To determine the duration of treatment required to observe increased editing, editing was also measured in hepatocytes following 1 or 2 days of insulin treatment compared with cells cultured without insulin. After 1 day, the fraction of edited apoB mRNA appeared increased relative to controls (Table 1), although this difference was not statistically significant ($P = .08$). After 2 days of treatment, the effect was essentially maximal (Table 1), indicating that apoB mRNA editing does not require the long-term (5-day) insulin exposure used in earlier experiments. Despite a documented decline in insulin binding and receptor autophosphorylation following chronic insulin treatment,[26,27] stimulation of editing did not diminish even after 5 days of exposure to insulin (Table 1).

Conceivably, enhanced steady-state levels of the edited apoB mRNA could result from increased activity and/or amounts of the components of the apoB editing complex, including the catalytic subunit, apobec-1.[10] Therefore, we measured steady-

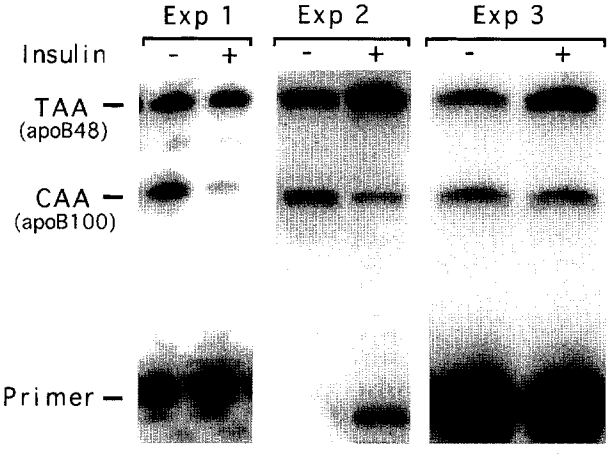


**Fig 1. Primer extension analysis of apoB mRNA editing in hepatocytes cultured in the absence or presence of insulin. Rat hepatocytes were cultured for 5 days in the absence or presence of bovine insulin (400 ng/mL). The extent to which hepatocyte apoB mRNA had undergone editing was determined by primer extension as described in the Methods. TAA and CAA refer to the sequence at codon 2153 of the apoB mRNA. CAA is the original, unedited sequence, while UAA is the edited sequence (TAA after PCR amplification). The results from three independent hepatocyte preparations are shown (Exp 1-3). Radiolabeled primer was used in excess for all experiments. In experiment 2, the gel was torn such that the primer band in the left lane is not visible.**

**Table 1. Effect of Insulin on ApoB mRNA Editing**

| Days in Culture | % Edited | |
|---|---|---|
| | − Insulin | + Insulin |
| 1 | 51 ± 4 | 65 ± 9 |
| 2 | 54 ± 4 | 74 ± 6 |
| 5 | 55 ± 7 | 78 ± 8 |

NOTE. Rat hepatocytes were cultured in the absence or presence of 400 ng/mL of insulin for 1, 2, and 5 days. ApoB mRNA editing was determined by primer extension as in the Methods. Results are the mean ± SD from 3 to 5 experiments, each using hepatocytes from a separate liver.

state levels of apobec-1 mRNA in control and chronically hyperinsulinemic cells. Because this mRNA is in low abundance and difficult to detect by Northern analysis, we used a RT-PCR assay to measure apobec-1 mRNA. A fragment of the apobec-1 mRNA was amplified by RT-PCR using several concentrations of cDNA from primary hepatocytes cultured for 5 days in the absence or presence of insulin (400 ng/mL). Amplification of a cDNA encoding the ribosomal protein L-32 or the housekeeping gene GAPDH was also performed in parallel using separate tubes to normalize for sample sizes. Figure 2A shows the ethidium bromide–stained bands derived from apobec-1 mRNA in a representative experiment, along with those from the mRNA of L-32. The reactions, performed in triplicate, show that the PCR products increased as the cDNA template for the reactions was increased. The apobec-1 product generated from the cDNA of insulin-treated hepatocytes (lanes 10 to 18) was clearly greater than that generated from control hepatocytes (lanes 1 to 9). Amplification of L-32 mRNA, on the other hand, was not affected by insulin. The digitized data in Fig 2A are plotted in Fig 2B, demonstrating the linearity of this assay over several concentrations of cDNA. The results of RT-PCR demonstrated an average twofold increase in the relative abundance of apobec-1 mRNA following insulin treatment (Table 2), indicating that insulin regulates apoB mRNA editing, at least in part, by altering expression of the apobec-1 gene.

RNase protection assays, using cyclophilin to normalize for sample size, were also performed to confirm that apobec-1 mRNA levels are elevated in response to insulin (Fig 3). Quantitation of the results by densitometry showed a 1.1-fold increase in apobec-1 mRNA abundance after 1 day of insulin treatment, while 2-day and 5-day insulin treatments resulted in 2.6- and 2.4-fold increases, respectively, consistent with the increased apoB mRNA editing observed at those time points and similar in magnitude to the increase detected using RT-PCR (Table 2).

## DISCUSSION

We previously observed in primary hepatocyte cultures that insulin treatment results in increased editing of apoB mRNA and increased secretion of apoB48.[14] The current study demonstrates that increased editing of apoB mRNA is accompanied by increased steady-state levels of mRNA for the catalytic subunit of the editing complex, apobec-1. The time course of the effect of insulin on apoB mRNA editing and on apobec-1 mRNA abundance is similar. These data indicate that increased editing of apoB mRNA in response to insulin is mediated, at least in part, through increased expression of apobec-1. In our previous studies, we observed that, in addition to a preferential increase in secretion of apoB48, there was an overall increase in apoB secretion.[14] This increase occurred in the presence of stable apoB mRNA abundance. Increased editing in the face of stable apoB mRNA levels would be expected to shift apoB production to the shorter isoform apoB48, but would not necessarily increase overall apoB secretion. Therefore, insulin may increase apoB secretion overall through other mechanisms, including increasing apoB translation or by promoting recruitment of apoB into VLDL, as has been observed by Coussons et al for oleic acid.[28]

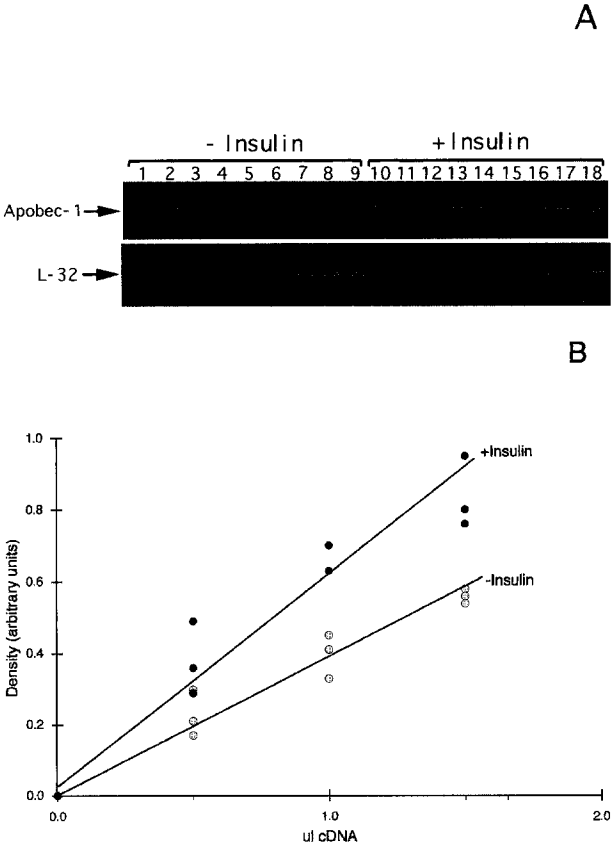


**Fig 2. Measurement of apobec-1 mRNA by RT-PCR. RT-PCR was performed on total cellular RNA isolated from rat hepatocytes cultured for 5 days in the absence or presence of bovine insulin (400 ng/mL) using primers specific for apobec-1. Assays were performed in triplicate with 3 increasing concentrations of template cDNA (0.5, 1.0, and 1.5 μL added to the reaction). Ethidium bromide–stained DNA bands in agarose gels of the PCR products were digitized and quantitated using NIH Image software. Sample sizes were normalized by performing parallel amplifications in separate tubes with primers specific for the ribosomal protein L-32. (A) Ethidium bromide–stained gel of a representative experiment. Insulin treatment is indicated above the lanes. Lanes 1-3 and 10-12 used 0.5 μL of cDNA template. Lanes 4-6 and 13-15 used 1.0 μL of cDNA template. Lanes 7-9 and 16-18 used 1.5 μL of cDNA template. The third measurement at 1.0 ul cDNA (+ Insulin), shown in lane 15, did not produce any product and was excluded as an outlier. (B) Plot of the quantitated density of the apobec-1 bands in A normalized to L-32. Dark circles represent values derived from cells cultured with insulin, while shaded circles represent values obtained using cells cultured without insulin. The best line through each set of points, determined by linear regression, is also shown.**

**Table 2. Effect of Insulin on Apobec-1 mRNA Abundance**

| Experiment | Density (arbitrary units) − Insulin | Density (arbitrary units) + Insulin | − Fold Increase |
|---|---|---|---|
| 1* | 0.30 ± 0.02 | 0.64 ± 0.04 | 2.1 |
| 2 | 0.60 ± 0.01 | 1.34 ± 0.24 | 2.2 |
| 3 | 0.40 ± 0.02 | 0.66 ± 0.05 | 1.6 |

NOTE. RT-PCR amplification of a fragment of the apobec-1 mRNA from hepatocytes grown 5 days in the absence and presence of 400 ng/mL insulin. Results expressed in arbitrary units assigned from densitometry of the PCR products resolved by electrophoresis as in Methods. Values are the mean ± SD of triplicate measurements normalized for sample size using mRNA for the ribosomal protein L-32 amplified in parallel with apobec-1.

*Experiment 1 values calculated from duplicate measurements normalized against GAPDH.

Unlike the situation in small intestinal cells of the adult rat and human, where editing of apoB mRNA is constant and nearly complete,[12] editing in rat hepatocytes is sensitive to hormonal and nutritional states,[9-13] including insulin treatment.[14] The observed increase in steady-state apobec-1 mRNA levels in response to insulin raises the possibility that insulin increases apobec-1 gene transcription, as suggested by the recent discovery of an insulin-response element in the 5′-flanking region of the rat apobec-1 gene.[25] However, it bears emphasis that enhancement of apobec-1 mRNA processing or increased stability of its mRNA cannot be ruled out and formal proof of the mechanism involved in the increased mRNA accumulation will await further study. Since apobec-1 activity is limiting for apoB mRNA editing in liver,[5,25] increased apobec-1 protein resulting from the elevated apobec-1 mRNA is likely to mediate the enhancement of apoB mRNA editing in rat hepatocytes after long-term hyperinsulinemia.

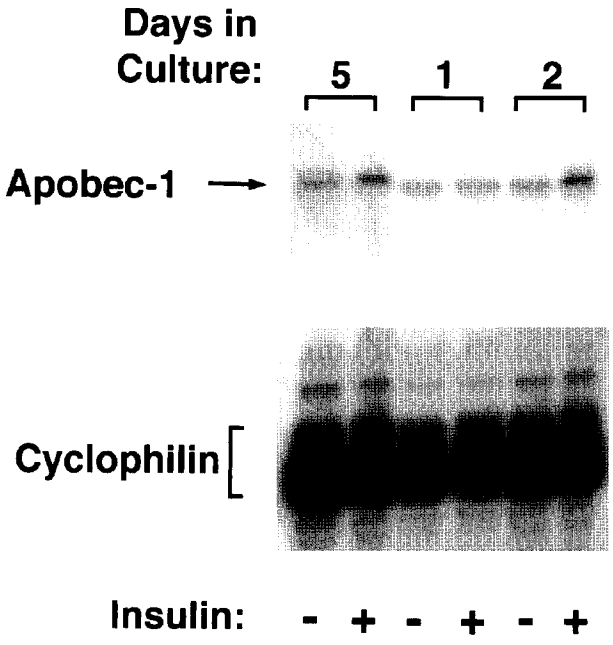


**Fig 3. Measurement of apobec-1 mRNA by RNase protection assay. RNase protection assays were performed on total cellular RNA isolated from rat hepatocytes cultured for the number of days indicated in the absence or presence of bovine insulin (400 ng/mL). Shown are the protected fragments for apobec-1 and cyclophilin, included for normalizing sample loading.**

Previous in vivo studies also support a potential role for insulin in modulating apoB mRNA editing: in intact rat studies, obese, hyperinsulinemic rats displayed a higher serum B-48/B-100 ratio.[19,29] The more recent of these studies also demonstrated a concomitant increase in the proportion of edited apoB mRNA in the liver,[19] as did Phung et al[18] in obese Zucker rat livers relative to lean controls. Phung et al also observed a 1.8-fold increase in the abundance of apobec-1 mRNA by Northern analysis in the livers of obese rats, similar to what we report here. Additionally, Funahashi et al[30] found greatly increased hepatic apoB mRNA editing 24 hours after refeeding following a 48-hour fast in normal rats, along with a parallel rise in the abundance of apobec-1 mRNA. In view of the fact that insulin alone can increase apoB mRNA editing, as well as apobec-1 mRNA abundance, one could argue that the insulin response following refeeding, as well as the hyperinsulinemia associated with insulin resistance, may be responsible for both the elevated apobec-1 mRNA and increased apoB mRNA editing seen in vivo under those conditions.

While it would be desirable to confirm that levels of apobec-1 protein are elevated along with its mRNA in response to insulin, physiologic levels of the protein have yet to be detected by immunoblot analysis or immunoprecipitation, presumably due to its low abundance. However, in vitro assays have demonstrated a correlation between the in vitro editing activity in liver cell extracts and the proportion of edited apoB mRNA in those same cells,[12,31] indicating that apobec-1 protein amount or activity is regulated in parallel with apoB mRNA editing. Interestingly, both ethanol and thyroid hormone administration have been shown to enhance hepatic apoB mRNA editing without affecting apobec-1 mRNA abundance,[11,12] suggesting that other mechanisms independent of apobec-1 mRNA levels, such as increased translation of apobec-1 mRNA or increased stability of the protein, may also be important in modulating apoB mRNA editing. Alterations in apoB mRNA editing may also reflect changes in the abundance of the complementation factors, a possibility that has yet to be formally examined. Another potential mechanism is suggested by the primary sequence of apobec-1 itself, which contains consensus sequences for cyclic adenosine monophosphate (cAMP)-dependent protein kinase, casein kinase, and protein kinase C.[32] Thus, in addition to controlling the quantity of apobec-1 in liver cells, regulation of hepatic apolipoprotein B mRNA editing may also involve modulating the activity of apobec-1 by altering its phosphorylation status. We are presently investigating the potential role of phosphorylation in regulating apobec-1 activity.

Our observation that apoB mRNA editing is stimulated by insulin is consistent with reports that, in rats, refeeding after a fast rapidly increases the fraction of edited hepatic apoB mRNA.[9,30] In contrast to the decline in the inhibitory effect of insulin on apoB secretion,[14,33] apoB mRNA editing did not become diminished with prolonged insulin exposure. This adds to the accumulating evidence that, in the hepatocyte, long-term hyperinsulinemia does not lead to a uniform attenuation of all insulin-mediated events.[34] Instead, in affected cells, the differ-

ent insulin-sensitive processes display individual and characteristic changes in how they respond to chronic hyperinsulinemia. The basis for the observed differential response of apoB mRNA editing (this study and Thorngate et al[14]) and the inhibition of apoB secretion[14,33] following prolonged versus acute insulin exposure is unknown, but may involve distinct pathways that diverge downstream of insulin receptor binding. This would allow for one of these processes to diminish after 5 days of insulin treatment and the other process to continue unabated.

In summary, our data show that insulin in rat hepatocytes increases steady-state levels of mRNA for apobec-1, the rate-limiting catalytic subunit of the apoB mRNA editing complex. Furthermore, the time courses of upregulation of apobec-1 mRNA abundance and apoB mRNA editing are similar.

## REFERENCES


1. Yao Z, Mcleod RS: Synthesis and secretion of hepatic apolipoprotein B-containing lipoproteins. Biochim Biophys Acta 1212:152-166, 1994
2. Sehayek E, Eisenberg S: The role of native apolipoprotein B-containing lipoproteins in atherosclerosis: Cellular mechanisms. Curr Opin Lipidol 5:350-353, 1994
3. Hodges P, Scott J: Apolipoprotein B mRNA editing: A new tier for the control of gene expression. Trends Biochem Sci 17:77-81, 1992
4. Greeve J, Altkemper I, Dieterich J, et al: Apolipoprotein B mRNA editing in 12 different mammalian species: Hepatic expression is reflected in low concentrations of apoB-containing plasma lipoproteins. J Lipid Res 34:1367-1383, 1993
5. Teng B, Blumenthal S, Forte T, et al: Adenovirus-mediated gene transfer of rat apolipoprotein B mRNA-editing protein in mice virtually eliminates apolipoprotein B-100 and normal low density lipoprotein production. J Biol Chem 269:29395-29404, 1994
6. Yamanaka S, Balestra ME, Ferrell LD, et al: Apolipoprotein B mRNA editing protein induces hepatocellular carcinoma and dysplasia in transgenic animals. Proc Natl Acad Sci USA 92:8483-8487, 1995
7. Hughes SD, Rouy D, Navaratnam N, et al: Gene transfer of cytidine deaminase apobec-1 lowers lipoprotein (a) in transgenic mice and induces apolipoprotein B editing in rabbits. Hum Gene Ther 7:39-48, 1996
8. Kozarsky KF, Bonen DK, Giannoni F, et al: Hepatic expression of the catalytic subunit of the apolipoprotein B mRNA editing enzyme (apobec-1) ameliorates hypercholesterolemia in LDL receptor-deficient rabbits. Hum Gene Ther 7:943-957, 1996
9. Baum CL, Teng BB, Davidson NO: Apolipoprotein B messenger RNA editing in the rat liver: Modulation by fasting and refeeding a high carbohydrate diet. J Biol Chem 265:19263-19270, 1990
10. Davidson NO, Carlos RC, Drewek MJ, et al: Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone. J Lipid Res 29:1511-1522, 1988
11. Lau PP, Cahill DJ, Zhu H, et al: Ethanol modulates apolipoprotein B mRNA editing in the rat. J Lipid Res 36:2069-2078, 1995
12. Inui Y, Giannoni F, Funahashi T, et al: REPR and complementation factor(s) interact to modulate rat apolipoprotein B mRNA editing in response to alterations in cellular cholesterol flux. J Lipid Res 35:1477-1489, 1994
13. Seishima M, Bisgaier CL, Davies SL, et al: Regulation of hepatic apolipoprotein synthesis in the 17 α-ethinyl estradiol-treated rat. J Lipid Res 32:941-951, 1991
14. Thorngate FE, Raghow R, Wilcox HG, et al: Insulin promotes the biosynthesis and secretion of apolipoprotein B-48 by altering apolipoprotein B mRNA editing. Proc Natl Acad Sci USA 91:5392-5396, 1994
15. Patsch W, Franz S, Schonfeld G: Role of insulin in lipoprotein secretion by cultured rat hepatocytes. J Clin Invest 71:1161-1174, 1983
16. Sparks CE, Sparks JD, Bolognino M, et al: Insulin effects on apolipoprotein B lipoprotein synthesis and secretion by primary cultures of rat hepatocytes. Metabolism 35:1128-1136, 1986
17. Sparks JD, Sparks CE: Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. J Biol Chem 265:8854-8862, 1990
18. Phung TL, Sowden MP, Sparks JD, et al: Regulation of hepatic apolipoprotein B RNA editing in the genetically obese Zucker rat. Metabolism 45:1056-1058, 1996
19. Yamane M, Jiao S, Kihara S, et al: Increased proportion of plasma apoB-48 to apoB-100 in non–insulin-dependent diabetic rats: Contribution of enhanced apoB mRNA editing in the liver. J Lipid Res 36:1676-1685, 1995
20. Sparks JD, Zolfaghari R, Sparks CE, et al: Impaired hepatic apolipoprotein B and E translation in streptozotocin diabetic rats. J Clin Invest 89:1418-1430, 1992
21. Elam MB, Simkevich CP, Solomon SS, et al: Stimulation of in vitro triglyceride synthesis in the rat hepatocyte by growth hormone treatment in vivo. Endocrinology 122:1397-1402, 1988
22. Davis RA: Lipoprotein synthesis and secretion by cultured hepatocytes. Methods Enzymol 129:272-283, 1986
23. Wu JH, Semenkovich CF, Chen SH, et al: Apolipoprotein B mRNA editing. Validation of a sensitive assay and developmental biology of RNA editing in the rat. J Biol Chem 265:12312-12316, 1990
24. Driscoll DM, Wynne JK, Wallis SC, et al: An in vitro system for the editing of apolipoprotein B mRNA. Cell 58:519-25, 1989
25. Hirano K, Min M, Funahashi T, et al: Cloning and characterization of the rat apobec-1 gene: A comparative analysis of gene structure and promoter usage in rat and mouse. J Lipid Res 38:1103-1119, 1997
26. Patsch W, Gotto AM, Patsch JR: Effects of insulin on lipoprotein secretion in rat hepatocyte cultures. J Biol Chem 261:9603-9606, 1986
27. Arsenis G, Livingston JN: Alterations in the tyrosine kinase activity of the insulin receptor produced by in vitro hyperinsulinemia. J Biol Chem 261:147-153, 1986
28. Coussons PJ, Bourgeois CS, Wiggins D, et al: Selective recruitment of apoB-48 for the assembly of VLDL in rat triacylglycerol-enriched hepatocytes. Arterioscler Thromb Vasc Biol 16:889-897, 1996
29. Azain MJ, Fukuda N, Chao FF, et al: Contributions of fatty acid and sterol synthesis to triglyceride and cholesterol secretion by the perfused rat liver in genetic hyperlipidemia and obesity. J Biol Chem 260:174-181, 1985
30. Funahashi T, Giannoni C, DePaoli AM, et al: Tissue-specific, developmental and nutritional regulation of the gene encoding the catalytic subunit of the rat apolipoprotein B mRNA editing enzyme: Functional role in the modulation of apoB mRNA editing. J Lipid Res 36:414-428, 1995
31. Harris G, Smith HC: In vitro apolipoprotein B mRNA editing activity can be modulated by fasting and refeeding rats with a high carbohydrate diet. Biochem Biophys Res Commun 183:899-903, 1992
32. Teng BB, Burant CF, Davidson NO: Molecular cloning of an apolipoprotein B messenger RNA editing protein. Science 260:1816-1819, 1993
33. Björnsson OG, Duerden JM, Bartlett SM, et al: The role of pancreatic hormones in the regulation of lipid storage, oxidation and secretion in primary cultures of rat hepatocytes. Short- and long-term effects. Biochem J 281:381-386, 1992
34. Saltiel AR: Diverse signaling pathways in the cellular actions of insulin. Am J Physiol 270:E375-E385, 1996