Nutrient-Gene Expression

Plasma Apolipoprotein B-48, Hepatic Apolipoprotein B mRNA Editing and Apolipoprotein B mRNA Editing Catalytic Subunit-1 mRNA Levels Are Altered in Zinc-Deficient Rats1,2

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ABSTRACT Apolipoprotein B (apoB) exists as two major isoforms and serves as an obligatory component of lipid-rich plasma lipoprotein particles. Apolipoprotein B mRNA editing is a zinc-dependent, site-specific cytidine deamination that determines whether the apoB-100 or apoB-48 isoform is synthesized. The objective of this work was to examine whether dietary zinc levels affect apoB mRNA editing in vivo. Adult male Sprague-Dawley rats were randomly assigned to zinc-deficient (ZD, <0.5 mg Zn/kg diet), zinc-adequate (ZA, 30 mg Zn/kg diet) or zinc-replenished (ZDA, ZD rats fed the ZA diet for last 2 d) dietary groups for 18 d. The ratio of plasma apolipoprotein B-48 (apoB-48) to total apoB was significantly lower in zinc-deficient compared with zinc-adequate rats. Primer extension analysis indicated a modest but significant reduction in hepatic apoB mRNA editing in ZD rats compared with that of the ZA group. In ZD rats, hepatic apoB mRNA editing and the percentage of plasma apoB-48 to total apoB were not different from ZA rats. The mRNA abundance of hepatic apobec-1 (apoB mRNA editing catalytic subunit 1) was significantly lower in ZD and ZDA rats than in ZA rats. In summary, the plasma ratio of apoB-48 to total apoB protein as well as hepatic apoB mRNA editing and hepatic apobec-1 mRNA levels were reduced in rats consuming a zinc-deficient diet. These data suggest that one or more components of apoB metabolism may be influenced by dietary zinc status.


KEY WORDS: • rats • dietary zinc • apolipoprotein B • apobec-1 • apolipoprotein B mRNA editing

Two isoforms of apolipoprotein B (apoB) are coded for by the same gene by a novel mechanism in which apoB mRNA is edited by a cytidine to uridine conversion (Chen et al. 1987, Powell et al. 1987). Editing results in changing the first base of the codon CAA, encoding glutamine 2153, to UAA, an in-frame translational stop codon. Consequently, apoB-48 is approximately half the size of apoB-100 and is identical to the amino-terminal half of apoB-100. Because the apoB-48 protein does not contain the carboxy-terminal half of the apoB-100, which is recognized by the LDL receptor, apoB-48 is not taken up by LDL receptor-mediated endocytosis. Instead, lipoproteins containing apoB-48 are cleared more quickly than those containing apoB-100 by a distinct receptor-mediated pathway involving apolipoprotein E.

The editing reaction is an intranuclear event (Lau et al. 1991), catalyzed by an editing complex or “editosome” (Harris et al. 1993) consisting of a 27-kDa protein termed apoB mRNA editing catalytic subunit 1 (apobec-1) and other complementary protein factors (Lau et al. 1990, Navaratnam et al. 1993b). Assembly of the editing complex is directed by an 11-nucleotide “mooring” sequence, beginning five nucleotides downstream of the editing site in the apoB mRNA (Shah et al. 1991, Smith et al. 1991). Although apobec-1 represents the catalytic component of the reaction, several studies have shown that both apobec-1 and the complementary factors must be present for editing to occur (Anant et al. 1995, Teng et al. 1993). Apobec-1 is an RNA-specific cytidine deaminase that exists as a homodimer (Lau et al. 1994) and has sequence homology with other cytidine deaminase family members (Navaratnam et al. 1995). Homology is highly conserved within the zinc-coordinating region, and in vitro studies have shown apoB mRNA editing to be zinc dependent (Barnes and Smith 1993).

Although apoB metabolism and plasma cholesterol have been linked to the development of atherosclerosis, zinc may also play a role; however, the cause-and-effect relationship has not been established. In atherosclerotic patients, the abnormally low levels of zinc in the plasma or serum (Halstead and Smith 1970, Netsky et al. 1969) as well as in the aorta (Volkov 1963) indicate that zinc metabolism has been altered. Zinc deficiency can affect the level of plasma lipoproteins, particularly those containing apolipoprotein B. In zinc-deficient rats, the HDL cholesterol level was decreased (Koo and Williams

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Abbreviations used: apoB, apolipoprotein B; apobec-1, apolipoprotein B mRNA editing catalytic subunit 1; DEPC, diethyl pyrocarbonate; RT-PCR, reverse transcriptase-polymerase chain reaction; ZA, zinc-adequate diet; ZD, zinc-deficient diet; ZDA, zinc-replenished diet.
1981), and there was a decrease in apoA-I and apoC of HDL (Koo and Lee 1988). Lipid malabsorption and defective chylomicron formation have also been reported in zinc-deficient rats (Koo and Turk 1977). Moreover, a reduction in the apoB content of chylomicrons was observed in the lymph of zinc-deficient rats (Koo et al. 1987). Because of the adverse changes in lipoprotein metabolism detected in zinc-deficient rats and the prevalence of marginal zinc deficiency in certain subpopulations of the U.S. (Sandstead 1995), these studies were performed to examine how zinc deficiency affects specific aspects of apoB metabolism.

MATERIALS AND METHODS

Experimental animals and diets. Eight-week-old male Sprague-Dawley rats were randomly assigned to one of the following three dietary treatment groups: zinc-adequate (ZA; n = 8), zinc-deficient (ZD; n = 8), or zinc-replenished (ZDA; n = 4). The replenished group was fed the ZD diet for 2 d of treatment; then they were fed the ZA diet. Dietary treatment was for 18 d; rats consumed their respective diet ad libitum and had free access to distilled demineralized water. Food was removed from the cages 10 h before the rats were killed. Each rat was housed separately in stainless steel wire cages with a room temperature of 22–24°C and a 12-h light:dark cycle. Body weights and daily food intake were measured once per week during the last week. During the last week, body weights and food intake were measured three times. All procedures were approved by the Animal Care and Use Committee of the University of Arizona. The basal diet was purchased from Dyets (Bethlehem, PA) and was formulated according to the AIN-93-M rodent diet (Reeves et al. 1993) recommendations. The composition of the diet was modified specifically for the egg white–based diet (Reeves et al. 1993) and included the omission of zinc from the mineral mix. The zinc-adequate diet contained 30 mg Zn/kg diet added in the form of ZnCO3, whereas the zinc-deficient diet contained <0.5 mg Zn/kg diet as determined by HNO3 digestion and subsequent flame atomic absorption spectrophotometry (model 180–70, Hitachi, San Jose, CA).

Isolation of plasma lipoproteins and apolipoprotein quantitation. On the day of killing, the rats were anesthetized with ether, and blood was collected by cardiac puncture using a syringe containing 10 mg EDTA. Plasma was centrifuged at 1000 rpm for 1 h, and blood was collected by cardiac puncture using a syringe containing 10 mg EDTA. Plasma was centrifuged at 1000 rpm for 1 h, and then digested in concentrated HNO3 before zinc, copper and iron were measured after samples were diluted 10-fold in distilled-deionized water before zinc analysis. Copper was measured directly in the digested samples, whereas zinc and iron were measured after samples were diluted 10-fold in distilled-deionized water. Certified reference solutions (Fisher Scientific, Fair Lawn, NJ) for zinc, copper and iron were used to generate linear standard curves for quantitation of each mineral. In addition, bovine Liver Standard Reference (U.S. Department of Commerce, National Institute of Standards, Gaithersburg, MD) was also analyzed to validate experimental findings.

Isolation of total cellular RNA from liver and intestine. Total cellular RNA was isolated from rat liver and small intestine using TRIzol Reagent (Life Technologies, Grand Island, NY). At the end of the dietary treatment, rats were killed, and each rat liver was immediately perfused with 40 mL of ice-cold diethyl pyrocarbonate (DEPC)-treated PBS through the portal vein. The liver was then quickly removed and 0.2 g tissue was placed in 2 mL TRIzol for homogenization. To isolate intestinal RNA, the small intestine was removed and rinsed with DEPC-treated ice-cold PBS. Mucosal cells were scraped using a rubber cell scraper, collected and transferred to a tube before the addition of 2 mL of TRIzol Reagent. Isolation of RNA was performed as recommended by the manufacturer’s protocol.

Ribonuclease protection assays. Plasmids containing rat apoB and apobec-1 cDNA were kindly provided by Dr. Teng (Baylor College of Medicine, Houston, TX). A 470-bp region of apoB cDNA (nucleotides 6512–6982) was inserted into the Smal site of pGEM-3ZI (+) vector (Promega, Madison, WI). The plasmid was used as a template to produce a 307-bp fragment by polymerase chain reaction (PCR) using the primers M13R (5′-AGGAAACAGCTATGAGC- CATG-3′) and forward primer J1 (5′-CAGTATCATATCCGTTGAAATCT-3′). Antisense radiolabeled apoB RNA probe was synthesized using T7 RNA polymerase. The protected fragment was 211 bases in length and corresponded to nucleotides 6771–6982. For apobec-1, a 423-bp Smal/KpnI fragment was subcloned into pbLueScript II (KS+) (Stratagene, La Jolla, CA). PCR was performed using the primers M13R (5′-AGGAAACAGCTATGAGCCATG-3′), forward primer EPI (5′-GCCTCGTTCAATGGAATCCCTG-3′) and apobec-1 (5′-GAGGAAACAGCTATGAGCCATG-3′) antisense radiolabeled apoB-1 RNA probe that yielded a 258-bp protected fragment during RNase protection. Before preparing the RNA probes for apoB and apobec-1, sequences were confirmed by both restriction enzyme analysis and direct DNA sequencing. For cyclophilin, a rat cyclophilin antisense template was purchased from Ambion (Austin, TX) and was used as an internal control. Labeling of RNA probes was performed using a MAXscript In Vitro Transcription Kit (Ambion) and (α-32P)-UTP (NEN Life Science Products, Boston, MA) according to the manufacturer’s protocol. Translation products were electrophoresed on 5% polyacrylamide, 8 mol/L urea gels, and full-length transcripts were localized by autoradiography. Gel slices containing full-length transcripts were excised and the RNA probe was eluted from each gel slice. RNase protection assays were performed using the RPA II kit (Ambion) essentially as described by the manufacturer. Total cellular RNA (10 µg) was combined with the apobec-1 or apoB probe, and the cyclophilin probe. RNase protection assay products were fractionated on 6% polyacrylamide, 8 mol/L urea gels at 300 V for 1.5 h. Gels were dried for 2 h and exposed to X-ray films for subsequent autoradiography for several different times to ensure linearity and then analyzed by laser densitometry (Molecular Dynamics). The size of the protected fragments was compared with [32P]-labeled RNA markers (Ambion) and a full-length undigested probe.

ApoB mRNA editing assay. Reverse transcriptase (RT)-PCR was performed essentially as described by Giannoni et al. (1994). RNA samples used to measure apoB mRNA editing were digested with DNase I to remove any DNA contamination. Each digestion reaction mixture contained ~5 µg of RNA, 2 units of DNase I (25 µl/Worthington Biochemical, Freehold, NJ), 10 mmol/L DTT, 50 mmol/L Tris-HCl (pH 7.5), 10 mmol/L CaCl2, 10 mmol/L MgCl2, and 1 µL RNasin (40 U/µl; Promega) and was digested at 37°C for 90 min. RNA was then extracted with phenol/chloroform and precipitated with ethanol. Pellets were washed with ether, resolubilized in DEPC H2O, and RNA concentrations were determined by measuring absorbance at 260 nm. For RT-PCR, 200 µmol/L of each dNTP, 10X RT buffer (Perkin-Elmer, Norwalk, CT), 5 µmol/L DNA Polymerase (Perkin-Elmer), 1 µmol/L MnCl2, and 50 pmol of downstream primer (5′-TCCTCAGCAGATCAATGATTG-3′) were combined with DNase I-digested RNA (~125 ng). RNA samples were then heated to 70°C for 7 min; annealing was at 55°C for 5 min, and reverse transcription was performed at 70°C for 10 min. After reverse transcription, 80 µL of PCR buffer, which contained 6 µL of 25 mmol/L MgCl2, 50 pmol of upstream primer (5′-TCCTCAGCAGATCAATGATTG-3′) and 8 µL of 10X cehating buffer (Perkin-Elmer) was added. The reaction was incubated for 3 min at 95°C followed by 30 cycles of 95°C/30 s, 55°C/1 min and 72°C/1.5 min. For the last cycle, extension was performed for 10 min. PCR with these rat primers resulted in the amplification of a 281-bp

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Values are means and Duncan’s new multiple range test (Jaccard and Becker 1990).

m with 2 95°C for 5 min; after annealing at 70°C for 10 min, the mixture was
P, Rats fed the ZD diet had significantly
48 bases in length. Whereas the edited apoB-48 mRNA product was
43 bases in length, whereas the edited apoB-48 mRNA product was
and analyzed by laser densitometry (Molecular Dynamics). Primer
nol-precipitated and separated on 6% polyacrylamide, 8 mol/L urea
primer extension analysis, the annealing primer was 5
(Perkin-Elmer) as another means to test for the presence of DNA. For
kept on ice during reverse transcription to check for DNA contam-
purification kit (Qiagen, Valencia, CA) after size verification by
Oligonucleotides were purchased from NBI (National Biosciences, 
Plymouth, MN) and PCR products were purified with QIAquick PCR
fragment flanking the editing site and was verified by sequencing. 
Oligonucleotides were purchased from NBI (National Biosciences, 
Plymouth, MN) and PCR products were purified with QIAquick PCR
purification kit (Qiagen, Valencia, CA) after size verification by
agarose gel electrophoresis. A negative control for each sample was
kept on ice during reverse transcription to check for DNA contami-
Before replenishment 7.60 ± 0.28a
During replenishment 7.29 ± 0.20a
Body weight, g
Liver weight, g/100 g body wt
Heart weight, g/100 g body wt
Hematocrit, % packed cell volume

TABLE 1

<table>
<thead>
<tr>
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<th>ZA</th>
<th>ZD</th>
<th>ZDA</th>
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<tr>
<td>Daily food intake, g/100 g body wt</td>
<td>7.60 ± 0.28a</td>
<td>4.85 ± 0.39b</td>
<td>5.81 ± 0.44b</td>
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<tr>
<td>Before replenishment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During replenishment</td>
<td>7.29 ± 0.20a</td>
<td>4.84 ± 0.58b</td>
<td>5.13 ± 1.23b</td>
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<td>Body weight, g</td>
<td>270 ± 7a</td>
<td>227 ± 2b</td>
<td>233 ± 3b</td>
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<td>Liver weight, g/100 g body wt</td>
<td>2.69 ± 0.03a</td>
<td>2.53 ± 0.05b</td>
<td>2.71 ± 0.05a</td>
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<tr>
<td>Heart weight, g/100 g body wt</td>
<td>0.36 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.36 ± 0.15</td>
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<tr>
<td>Hematocrit, % packed cell volume</td>
<td>49.57 ± 0.80</td>
<td>51.13 ± 0.44</td>
<td>49.10 ± 0.41</td>
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</table>

1 Food intake values represent data from either before the 2-d replenishment phase or during the zinc replenishment.
2 Values are group means ± SEM, n = 8 (ZA and ZD) and n = 4 (ZDA group). Means in a row not sharing a letter are significantly different, P > 0.05.

RESULTS

Dietary intake, body and organ weights, and tissue mineral concentrations. Rats fed the ZD diet had significantly lower body weight and relative liver weight compared with those fed the ZA diet (Table 1). In contrast, the body weight of the ZDA rats was not significantly different from the ZD rats after consumption of the ZA diet for the last 2 d before killing (Table 1). However, the relative liver weight of the ZDA rats was not different from that of the ZA rats. Daily food intake of the rats fed a ZD diet was significantly lower than that of rats fed the ZA diet, suggesting that a reduction in food intake in rats fed the ZD diet may have contributed in part to the depressed weight gain (Table 1). Furthermore, the 2-d supplementation with the ZA diet did not increase food consumption of the ZDA compared with the ZD rats (Table 1).

Significantly lower plasma and hepatic zinc concentrations were observed in ZD rats compared with ZA rats, whereas plasma and hepatic zinc levels of the ZDA rats were not different from those of ZA rats (Table 2). No differences in hepatic copper levels were observed among the treatment groups. However, hepatic iron levels were significantly greater in ZD and ZDA rats compared with ZA rats (Table 2). Thus, the plasma and hepatic mineral analyses confirmed that the ZD rats were indeed deficient in zinc compared with controls.

Plasma apoB-48 to total apoB protein ratios. Samples of each of the apoB-100 and apoB-48 were chosen from each dietary treatment group and are shown in Figure 1A. Plasma apoB-48, apoB-100 and total apoB levels were not significantly different among the three dietary treatment groups (Fig. 1B). A ratio was calculated as the percentage of plasma apoB-48 to total apoB [apoB-48/(apoB-48 + apoB-100) × 100] to examine how the subtle differences in apoB-100 and apoB-48 concentrations among groups may affect the overall plasma apoB profile (Fig. 1C). The percentage of plasma apoB-48 to total apoB in the ZD rats was significantly reduced to nearly half the level observed in the ZA rats. In the ZDA group, zinc replen-

TABLE 2

<table>
<thead>
<tr>
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<th>ZA</th>
<th>ZD</th>
<th>ZDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma zinc, μmol/L</td>
<td>24.6 ± 0.6a</td>
<td>19.6 ± 0.6b</td>
<td>23.9 ± 0.5a</td>
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<td>Hepatic zinc, μmol/g wet wt</td>
<td>0.516 ± 0.013a</td>
<td>0.447 ± 0.004b</td>
<td>0.506 ± 0.007a</td>
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<tr>
<td>Hepatic copper, μmol/g wet wt</td>
<td>0.049 ± 0.001</td>
<td>0.046 ± 0.001</td>
<td>0.049 ± 0.001</td>
</tr>
<tr>
<td>Hepatic iron, μmol/g wet wt</td>
<td>2.57 ± 0.04b</td>
<td>3.19 ± 0.06a</td>
<td>3.15 ± 0.09a</td>
</tr>
</tbody>
</table>

1 Values are group means ± SEM, n = 8 (ZA and ZD) and n = 4 (ZDA group).
2 Group means in a row not sharing a letter are significantly different, P < 0.05.
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ApoB and apobec-1 mRNA abundance. In view of our findings that hepatic apoB mRNA editing was altered, hepatic apoB and apobec-1 mRNA levels were also measured. Representative samples of apoB mRNA from the three treatments are shown in Figure 3A and illustrate that there was no difference in hepatic apoB mRNA abundance among the three treatment groups. Analysis of apoB mRNA bands by laser densitometry, using cyclophilin mRNA as the internal standard, indicated that there was no difference in apoB mRNA abundance among the treatment groups (Fig. 3B). These data support the observation that total apoB plasma protein levels were not significantly different among groups. Because the plasma apoB-48 to total apoB ratio and hepatic apoB mRNA editing data showed differences among treatment groups, hepatic apobec-1 mRNA abundance was measured. Bands corresponding to apobec-1 mRNA among the experimental animals suggested that the highest abundance of apobec-1 mRNA was present in ZA rats (Fig. 4A). The hepatic apobec-1 mRNA abundance was significantly higher in ZA rats compared with ZD or ZDA rats (Fig. 4B). Apobec-1 mRNA abundance was reduced by 46 and 37% for the ZD and ZDA rats, respectively, compared with the ZA rats.

Hepatic and intestinal apoB mRNA editing. Representatives samples of primer extension products from the three treatments are shown in Figure 2A. Hepatic editing was significantly lower in ZD than in ZA rats. However, hepatic editing in the ZDA rats was significantly different only from the ZD rats (Fig. 2B). Analysis of intestinal editing levels was also performed; however, there was no significant difference in intestinal editing among the ZA, ZD and ZDA groups (data not shown). Mean value for intestinal editing for the three treatment groups was 86.9 ± 1.57%, which is similar to previously reported rat intestinal editing levels.
Reductions in food intake and growth, previously observed in zinc-deficient rats (Chesters and Quarterman 1970), were also observed in this study. We also included a third group that was comprised of ZD rats fed the ZA diet for the last 2 d before killing. This group (ZDA rats) was used as an attempt to evaluate the specific effect of zinc replenishment. Although plasma and hepatic zinc returned to control levels after the 2-d replenishment with ZA diet, the ZDA rats did not consume significantly more food than ZD rats during that period. Two days may simply not be enough time for intake to increase to normal levels. Perhaps there is a lag time before zinc-deficient rats resume normal growth after receiving a zinc-adequate diet. Nevertheless, 2 d of zinc replenishment did specifically cause hepatic zinc, the percentage in plasma of apoB-48 compared with total apoB and hepatic apoB mRNA editing levels to revert to those observed in ZA rats. In addition, all rats were food deprived for 10 h before being killed so that the presence or absence of food in the gastrointestinal tract would not have been a factor or potential difference among animals. We chose not to use a pair-fed group because this technique tends to inflict the constant stress of semistarvation, which may induce metabolic alterations as well as result in a meal-eating food...
consumption pattern compared with animals consuming food ad libitum. Therefore, the restriction in food intake imposed by pair-feeding may have limitations in mimicking the reduction in food intake associated with zinc deficiency. Although it can be argued that both pair-fed and zinc-repletion controls have certain shortcomings, we believe that zinc repletion offered an intriguing option in determining whether the effects were specific to zinc.

Moreover, in this study, the lack of change in hepatic copper concentrations among the three dietary treatment groups suggests that copper-zinc antagonism was not important. In the ZD rats, the stable hepatic copper level may indicate that the zinc deficiency was not drastic enough to alter hepatic zinc-copper equilibrium. Thus, the ZD rats may be only moderately deficient in zinc, which would more closely resemble the chronic marginally zinc-deficient status often encountered in certain human populations (Hambidge et al. 1985). Nevertheless, the hepatic iron concentrations were markedly higher in ZD rats than in ZA rats and remained elevated for the ZDA rats. Because iron as well as zinc are transported by transferrin in the portal circulation (Evans and Winter 1975), a reduction in dietary zinc may enhance the amount of iron bound to transferrin, leading to hepatic iron accumulation. However, normalized hepatic zinc levels in the zinc-repleted group did not result in a reduction in hepatic iron.

Impaired absorption of dietary lipids (Koo et al. 1986) as well as alterations in hepatic chylomicron apoB content have been reported in zinc-deficient rats (Koo et al. 1987). In contrast, in this study, the plasma total apoB, apoB-100 and apoB-48 levels were not significantly changed by dietary treatment. Nevertheless, we did observe a markedly reduced plasma apoB-48 to total apoB ratio in ZD rats. We chose to measure editing because apoB mRNA editing has been shown to be zinc dependent in vitro (Barnes and Smith 1993, Navaratnam et al. 1993a). We suspected that zinc status may have affected editing in these rats and that this may be related to the ratio of plasma apoB-48 to total apoB. Indeed, we first reported that hepatic editing was altered in zinc-deficient rats using the direct primer extension method.¹ These findings were confirmed using the RT-PCR primer extension method reported in the present studies. Both assays revealed that the percentage of hepatic apoB mRNA edited was decreased significantly in the ZD rats. Interestingly, in the ZDA rats, the percentage of plasma apoB-48 to total apoB as well as hepatic apoB mRNA editing returned to control levels.

However, because differences in plasma apoB-48 ratios between zinc-adequate and zinc-deficient rats were relatively large compared with the modest but significant differences in hepatic editing, we suspect that other facets of apoB metabolism may also be affected. Processes such as synthesis, degradation and peripheral uptake may also be altered by zinc status, but we chose to focus our efforts on editing for the current studies because other studies have also demonstrated that dietary factors can influence hepatic apoB mRNA editing. For example, when rats were food deprived for 24 h, the proportion of hepatic apoB mRNA editing was decreased to 30–40%. Yet, when the rats were refed a high carbohydrate diet for 24–48 h, editing was markedly increased to 80–90% (Baum et al. 1990). Similarly, chronic ethanol consumption in rats increased hepatic apoB mRNA editing to 90–100% (Lau et al. 1995). Moreover, hepatic apoB mRNA editing was increased when rats were fed a diet deficient in copper (Reaves et al. 1996). Even though dietary factors can alter hepatic apoB mRNA editing, intestinal apoB mRNA editing appears to be more resistant to modulation as indicated by these as well as numerous other studies. In addition, hepatic apoB mRNA abundance was not affected by the zinc status of the rats. Other studies have also shown that dietary modulation does not affect the hepatic total apoB mRNA abundance (Lau et al. 1995, Leighton et al. 1990, Nassir et al. 1996, Reaves et al. 1996).

A significant decrease in hepatic apoB-1 mRNA abundance in the ZD rats was observed in this study. In previous studies, changes in hepatic editing were found to correlate with changes in hepatic apoB-1 mRNA abundance. Rats that were food deprived for 24 or 48 h demonstrated a decrease in hepatic apoB-1 mRNA abundance and a concomitant decrease in hepatic apoB mRNA editing. After the refeeding of a high carbohydrate diet for 24 or 48 h, hepatic apoB-1 mRNA abundance was increased 2.1- to 2.8-fold, and hepatic editing of apoB mRNA was also increased (Funahashi et al. 1995). In contrast, cholesteryl ester supplementation depressed hepatic apoB mRNA editing and reduced apoB-1 mRNA abundance in rats (Inui et al. 1994). However, changes in editing are not always “coupled” with changes in apoB-1 mRNA abundance, illustrating that several mechanisms may play a role in regulating apoB editing. For example, in our ZD rats, the hepatic apoB mRNA editing level was normalized to that of the ZA rats, whereas apoB-1 mRNA remained depressed. These data suggest that a post-transcriptional regulatory mechanism could be responsible for the rapid normalization of editing levels in the ZDA rats before the restoration of the apoB-1 mRNA abundance to the level of controls. Obviously, the same mechanism could be responsible for the observed decrease in hepatic editing of the ZD rats, and it may not be simply transcriptional regulation of apoB-1 that is responsible for the alteration in editing.

It must be noted that data from our study differ from those recently reported by Nassir et al. (1996). There are many differences in the experimental design between the two studies, which may account for our incongruent observations. For example, our rats were slightly older and were sexually mature animals. We fed our rats AIN-96M rodent diet as opposed to their use of AIN-76A formulations. Although the duration of treatment was similar, our zinc-deficient diet was lower in zinc (<0.5 vs. 1 mg Zn/kg diet). Food deprivation or food deprivation and refeeding have both been shown to affect hepatic apoB mRNA editing levels in rats (Baum et al. 1990, Harris and Smith 1992, Leighton et al. 1990). These data suggest that there is a critical period of time just before killing in which dietary intake can influence editing levels; therefore, dietary intake should be very similar in animals of each treatment group during this time period. As an attempt to minimize these variations, we chose to remove food from the cages 10 h before killing. In the other study, food was not removed before killing, which potentially could be a key difference between the two studies. Also, instead of using pair-fed animals we opted for a zinc-replenishment group in an effort to show the effects of adding back zinc to the zinc-depleted rats. However, there were some similarities between studies; both studies found a reduction in plasma apoB-48 to total apoB in zinc-deficient vs. zinc-adequate rats. In addition, in their study, there appeared to be a trend for hepatic editing to be lower in ZD than ZA rats, but the differences were not significant. Also, both studies found that hepatic apoB mRNA abundance was not affected by the dietary treatments. Exactly how these differences in experimental design relate to the different observations in the two studies is uncertain and remains open to speculation.

In summary, we found that rats consuming a zinc-deficient diet exhibited a significantly reduced percentage of plasma...
apoB-48 to total apoB compared with zinc-adequate and zinc-repleted rats. A modest yet significant reduction in hepatic apoB mRNA editing was observed in zinc-deficient rats, but editing levels returned to control values in rats of the zinc-replenished group. Hepatic apoB mRNA abundance was reduced in zinc-deficient rats and was not restored by zinc replenishment. These data suggest that several aspects of apoB metabolism may be affected when utilizing a diet low in zinc to induce zinc deficiency.

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LITERATURE CITED


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