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Hepatic apolipoprotein B synthesis in copper-deficient rats

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The present study was designed to examine if induction of apolipoprotein B synthesis is associated with hypercholesterolemia in copper-deficient rats. This hypercholesterolemia mainly resides in an increase in the HDL-1 and LDL and is associated with a significant increase in plasma apoB concentration. Liver apoB mRNA levels were not significantly modified in deficient animals as compared to control rats. Studies on liver apolipoprotein synthesis indicated that apoB100 synthesis was increased in deficient animals whereas apoB48 synthesis was unchanged. Thus, it appears that the increase in apoB synthesis in the liver of copper-deficient rats occurs at the posttranscriptional level. The selective increase in apoB100 synthesis indicates the possible impact of this deficiency on the editing of apoB. An increase in apoB100 synthesis by the liver in copper-deficient rats may significantly contribute to the increase in plasma concentration of LDL.

Copper; Apolipoprotein B; Lipoprotein; Liver; Rat

1. INTRODUCTION

Recent findings provided considerable information on the influence of copper deficiency on lipid metabolism and the cardiovascular system [1,2]. Copper deficiency has been demonstrated to induce hypercholesterolemia and causes changes in lipoprotein concentration and composition [2–4]. In copper-deficiency, increase in plasma lipid and apolipoprotein contents resides mostly in HDL for the rat model, whereas in rabbits and humans this increase is associated mainly with LDL [1,2]. Available evidence suggests that hypercholesterolemia in copper-deficient rats may be the result of an increased rate of cholesterol synthesis in the liver [2,5]. ApoB is an obligatory component of lipoproteins synthesized by the liver and serves as recognition marker for the uptake of LDL by the apoB, -E receptors [6]. An increased apoB synthesis is expected to accompany the elevated cholesterol synthesis in order to maintain an enhanced lipoprotein secretion. However, at present there is no direct evidence available to support the proposed induction of apolipoprotein synthesis by copper deficiency [2]. The present study was then designed to examine apoB synthesis for better understanding the mechanism responsible for hyperlipemia associated with copper deficiency.

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Abbreviations: apo, apolipoprotein; CM, chylomicrons; EDTA, ethylenediamine tetraacetic acid; ELISA, enzyme-linked immunoassay; HDL, high density lipoproteins; LDL, low density lipoproteins; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenyl methyl sulfonyl fluoride; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; VLDL, very low density lipoproteins.

2. MATERIALS AND METHODS

2.1. *Animals and diets*

Weanling male Wistar rats (Iffa-Credo, L'Arbresle, France) were divided at random into copper-deficient and control groups. Rats were housed in wire-bottomed cages in a temperature-controlled room (22°C) with 12 h light–dark cycle and were pair-fed the appropriate diets for 6 weeks. The semi-purified diets containing (g/kg) casein 200, sucrose 650, corn oil 50, alphacel 50, DL-methionine 3, choline bitartrate 2, modified AIN-76 mineral mix 35, AIN 76A vitamin mix 10 (ICN Biomedicals, Orsay, France). Cupric carbonate was omitted from the AIN-76 mineral mix in the copper-deficient diet. The copper concentrations of diet were 0.4 mg/kg (deficient) and 6 mg/kg (control). Fed animals were anesthetized with sodium pentobarbital (40 mg/kg body weight i.p.) and then killed. Blood was collected into tubes containing EDTA and plasma was obtained by low-speed centrifugation (2,000 × *g*). Hematocrit was determined by centrifugation in a capillary tube system to obtain packed cells.

2.2. *Plasma analyses*

Triglycerides (Biotrol, Paris, France) and cholesterol (BioMérieux, Charnonnières-les-Bains, France) were determined in plasma by enzymatic procedures. Noncompetitive ELISA was used to measure apoB in plasma as described previously [7].

2.3. *ApoB distribution in lipoprotein fractions*

Lipoprotein fractions were separated from plasma by continuous gradient ultracentrifugation in a L8.70 Beckman centrifuge using a SW41 rotor as described previously [8]. A control tube containing only saline solutions was used to determine the density of fractions by refractometry. 22 fractions were collected from the meniscus of the tubes and total protein content in these fractions was assayed [9]. Aliquots of isolated fractions were submitted to PAGE-SDS on 5% acrylamide slab gel. The samples were subsequently electroblotted to nitrocellulose membranes (Hybond-C Super, Amersham, Bucks, UK). The distribution of apoB in lipoprotein fractions was shown by immunostaining of apoB with sheep anti-rat apoB immunoserum and

peroxidase-labeled rabbit anti-sheep IgG (Sigma, L'Isle d'Abeau, France).

2.4. *RNA extraction and analysis*

Total cellular RNA was isolated from liver tissue using the guanidinium/phenol/chloroform method according to Chomczynski and Sacchi [10]. RNA was quantitated by measuring the absorbance at 260 nm. Its integrity was assessed by agarose-gel electrophoresis and visualization of 18S and 28S ribosomal RNAs by ethidium bromide staining. Aliquots of total RNA were subjected to quantification of mRNA content by dot-blot analysis on nylon filters. Hybridization of immobilized RNA to cDNA probes for rat apoB (kindly provided by P. Cardot) and for mouse 18S (kindly provided by G. Veyssière) labeled with [α-^{32}P]dATP (Amersham) and washing was conducted as previously described [11]. The filters were blotted dry, and autoradiography was performed with intensifying screens at −70°C. Quantification of specific mRNA was performed by densitometric analysis of the hybridization signal using a laser densitometer (Ultroscan XL, LKB, Sweden). Relative abundance of the apoB mRNA was expressed as the apoB/18S mRNA ratio.

2.5. *Determination of hepatic apoB synthesis rates*

ApoB synthesis rates were determined in the liver of copper-adequate and copper-deficient rats as described by Baum et al. [12]. Animals were anesthetized with sodium pentobarbital and received 1.0 mCi L-[4,5-^{3}H]leucine (specific activity 5.29 TBq/mmol; Amersham) via intraportal vein injection. Following a 15 min incubation period, animals were exsanguinated and the liver perfused in situ for 15 min with 100 ml of ice-cold PBS, 20 mM leucine containing freshly added PMSF (1 mM final concentration). Livers were quickly removed, weighed, and portions removed for analysis of apoB synthesis rates. Pieces were taken from all lobes for homogenization in PBS, 1% Triton, 2 mM leucine containing freshly added protease inhibitors (PMSF 1 mM, benzamidine 1 mM, *N-p*-tosyl-L-lysine chloromethyl ketone 25 μM, leupeptin 100 μM, EDTA 5 mM, aprotinin 450 μM, pepstatin 2 μM). A 225,000 × *g* supernatant was prepared and stored at −80°C prior to immunoprecipitation. Aliquots of homogenates were saved for measurement of total protein concentration and TCA-insoluble radioactivity. Quantitative immunoprecipitation of apoB was performed using monospecific polyclonal rabbit antiserum directed against rat apoB. Immunoprecipitation was conducted using a buffer with the following final composition: 100 mM NaCl, 50 mM LiCl, 5 mM EDTA, 50 mM Tris, 0.02% sodium azide, 0.5% Triton, 0.5% sodium deoxycholate, an 0.05% SDS, at pH 7.4 and washed *S. aureus* cells (Pansorbin, Calbiochem, San Diego, CA). Immune complex was washed extensively and separated on denaturing SDS-PAGE disc gels (4% acrylamide). Gels were sliced and radioactivity was determined by liquid scintillation spectrometry (Kontron, St-Quentin-en-Yvelines, France) following addition of 3% Protosol-Econofluor (NEN, Boston, MA) to gel slices. Data are expressed as percent of total (TCA-insoluble) radioactivity.

2.6. *Statistics*

Values are given as means ± S.E.M. Data were analyzed by Student's *t*-test.

3. RESULTS

As shown in Table I, copper-deficient rats had lower body weights and higher relative liver and heart weights than control rats. Reduced plasma copper concentrations and reduced hematocrit values were found in copper-deficient animals. Plasma cholesterol and apoB concentrations were significantly greater in copper-deficient rats as compared to control animals.

Density gradient ultracentrifugation study indicates marked alteration in the distribution of lipoproteins in copper-deficient rats as compared to control ones (Fig. 1). Hypercholesterolemia associated with copper deficiency leads to an increase in HDL and LDL protein concentrations. As shown by immunoblot analysis of isolated lipoprotein fractions (Fig. 2), the increase in plasma apoB levels in copper-deficient rats mostly results from an increase in apoB100 in LDL.

Table I

Body and organ weights, and biochemical data of control and copper-deficient rats

	Control	Copper-deficient
Body weight (g)	289 ± 3	242 ± 8**
Relative liver weight (g/100 g)	3.8 ± 0.1	6.1 ± 0.2**
Relative heart weight (g/100 g)	0.33 ± 0.01	0.73 ± 0.05**
Hematocrit (volume fraction)	0.41 ± 0.02	0.17 ± 0.03**
Plasma copper (μmol/l)	14.6 ± 0.50	0.60 ± 0.08**
Plasma triglycerides (mmol/l)	0.70 ± 0.09	0.98 ± 0.15
Plasma total cholesterol (mmol/l)	1.55 ± 0.06	1.98 ± 0.11*
Plasma apoB (mg/l)	65 ± 4	115 ± 6**

Results are mean ± S.E.M. of 12 rats per group. *$P < 0.01$; **$P < 0.001$.

There was no difference in total protein synthesis in both groups as shown by [^{3}H]leucine incorporation into total TCA-insoluble material (control 118 ± 10 cpm/μg protein ($n = 6$); copper deficient 110 ± 13 cpm/μg protein ($n = 6$) $P > 0.05$). Studies of apoB synthesis in the liver have shown (Table II) that there is a marked (doubling) increase in hepatic apoB100 synthesis without modification in apoB48 synthesis in copper-deficient rats as compared to control ones. Copper-deficiency did not modify apoB mRNA level in the liver (Table II).

4. DISCUSSION

Several measurements are indicators of dietary copper deficiency in addition to the reduced plasma copper concentration, such as decreased body weight gain, increased heart- and liver-to-body weight ratio and lower hematocrit [13]. Copper deficiency increases blood cholesterol more consistently than it increases blood triglyceride concentrations [1,2]. This hypercholesterolemia is mainly the result of an increase in the concentration of HDL and in particular in HDL1 subfraction [2,4]. Additionally, as shown in the present work by density gradient ultracentrifugation study and immunoblot analysis of isolated fractions, the increase in plasma apoB concentration in copper-deficient rats mostly results from an increase in LDL concentration.

The present study demonstrates that increased apoB synthesis in the liver accompanies copper deficiency induced hyperlipemia. Moreover, specific increase in

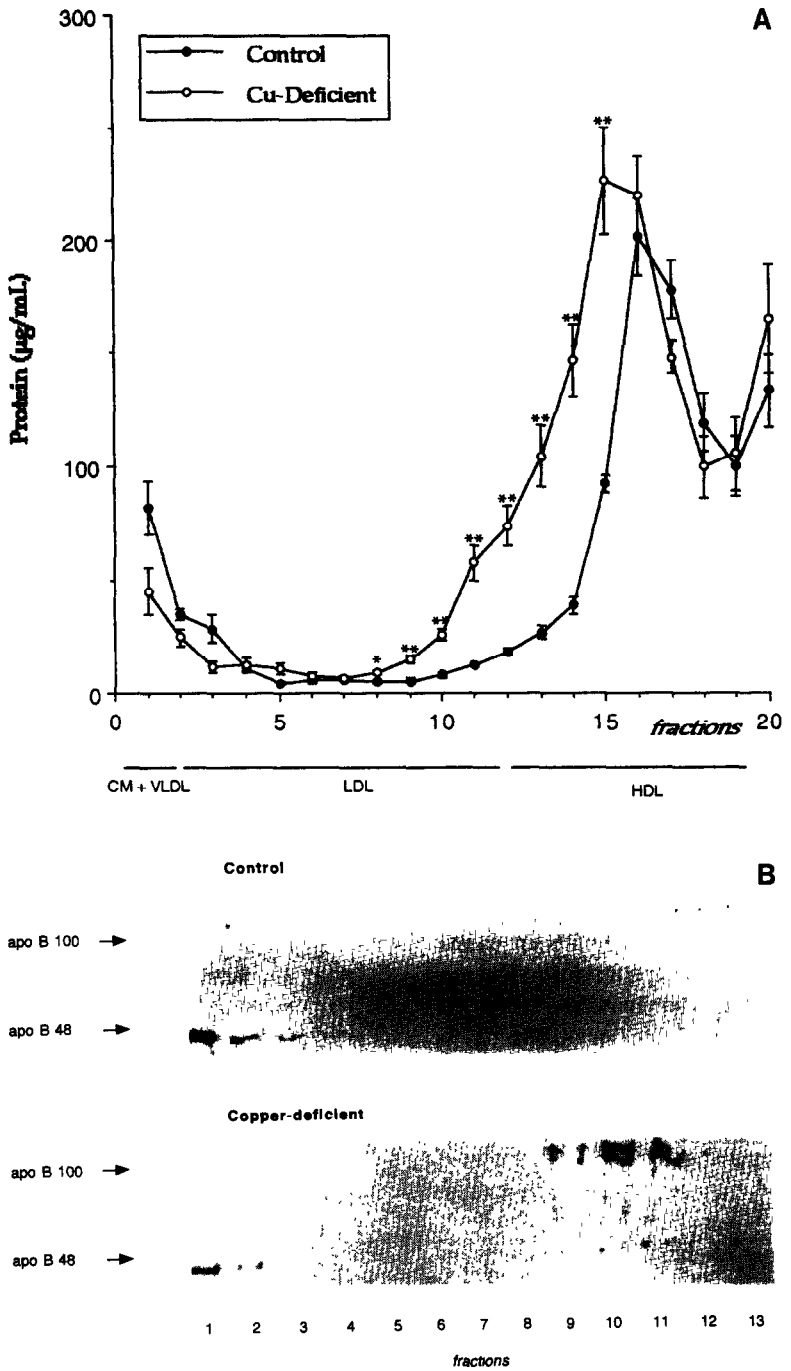

Fig. 1. (A) Protein distribution in lipoprotein fractions separated by density gradient ultracentrifugation from control (n = 6) or copper-deficient rats (n = 5). Results are expressed as mean ± S.E.M.; $P < 0.01$; $P < 0.001$. (B) Immunoblot analysis of apolipoprotein B distribution in lipoprotein fractions separated by density gradient ultracentrifugation from control or copper-deficient rats.

apoB100 synthesis suggests that this may contribute to the increase in plasma concentration of LDL containing essentially apoB as apolipoprotein. Increases in the synthetic rates of fatty acids and an accelerated efflux of newly synthesized cholesterol ester from the liver to the plasma have been observed in copper deficiency [2]. These observations suggest that lipoprotein secretion by the liver is enhanced in copper-deficient rats. Thus, the induction of apoB synthesis by copper deficiency may be related to this increase in production of fatty acids and cholesterol in the liver in order to both assemble and secrete triglyceride-rich lipoproteins.

Control of apoB synthesis and secretion can occur at number of levels involving changes in apoB mRNA

Table II

Apolipoprotein B synthesis and mRNA level in the liver of control and copper-deficient rats

	Control	Copper-deficient
ApoB100 synthesis (% of total protein synthesis)	0.20 ± 0.03	0.41 ± 0.04*
ApoB48 synthesis (% of total protein synthesis)	0.15 ± 0.03	0.21 ± 0.04
ApoB mRNA level (% of control)	100 ± 11	82 ± 12

Results are mean ± S.E.M. of 6 rats per group. *$P < 0.01$.

abundance, apoB mRNA editing, and through modifications in intracellular degradation of newly synthesized apoB [6,14]. In agreement with our previous study [11] we have shown that copper deficiency is not associated with modification in apoB mRNA in the liver. Thus it appears that copper deficiency affects apoB synthesis at the post-transcriptional level. Unlike the humans synthesizing only one form of apoB (apoB100), rat liver synthesizes two forms: apoB100 and apoB48. The mechanism accounting for the synthesis of these two forms of apoB involves mRNA editing [15]. It has been shown that the relative proportions of apoB100 and apoB48 made by the liver are not constant and may be physiologically regulated [12]. Recent studies have demonstrated that hepatic apoB mRNA editing in the rat is regulated developmentally and by various nutritional and hormonal factors [12,16]. It has been shown that apoB mRNA abundance and apoB editing are regulated dramatically by the food deprivation and refeeding [12]. As a precautionary measure, in our study we equalized dietary intake by daily pair-feeding in attempt to counteract the possible effect of differences in food intake on the studied parameters between control and deficient animals. In summary, it appears that the increase in apoB synthesis in the liver of copper-deficient rats occurs at the posttranscriptional level. The selective increase in apoB100 synthesis indicates the possible impact of this deficiency on the editing of apoB. An increase in apoB100 synthesis by the liver in copper-deficient rats may significantly contribute to the increase in plasma concentration of LDL, containing essentially apoB100 as apolipoprotein.

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REFERENCES

[1] Lei, K.Y. (1990) in: Role of Copper in Lipid Metabolism (K.Y. Lei, ed.) pp. 1–23, CRC Press, Boca Raton.
[2] Lei, K.Y. (1991) Annu. Rev. Nutr. 11, 265–283.
[3] Lefevre, M., Keen, C.L., Lönnerdal, B., Hurley, L.S. and Schneeman, B.O. (1986) J. Nutr. 116, 1735–1746.
[4] Al-Othman, A.A., Rosenstein, F. and Lei, K.Y. (1992) J. Nutr. 122, 1199–1204.
[5] Young, N.Y., Carr, T.P., McNamara, D.J. and Lei, K.Y. (1991) Biochim. Biophys. Acta 1082, 79–84.
[6] Gibbons, G.F. (1990) Biochem. J. 268, 1–13.
[7] Rayssiguier, Y., Gueux, E., Bussière, L. and Mazur, A. (1993) J. Nutr. (in press).
[8] Sérougne, C., Férézou, J. and Rukaj, A. (1987) Biochim. Biophys. Acta 921, 522–530.
[9] Markwell, M.A.K., Hass, S.M., Bieber, L.L. and Tolbert, N.E. (1978) Anal. Biochem. 87, 206–210.
[10] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[11] Mazur, A., Nassir, F., Gueux, E. and Cardot, P. (1992) Biol. Trace Element Res. 34, 107–113.
[12] Baum, C.L., Teng, B.-B. and Davidson, N.O. (1990) J. Biol. Chem. 265, 19263–19270.
[13] Prohaska, J.R. (1990) J. Nutr. Biochem. 1, 453–46??.
[14] Sparks, J.D., Zolfaghari, R., Sparks, C.E., Smith, H.C. and Ficher, E.A. (1992) J. Clin. Invest. 89, 1418–1430.
[15] Hodges, P. and Scott, J. (1992) Trends Biochem. Sci. 17, 77–81.
[16] Inui, Y., Hausman, A.M.L., Nanthakumar, N., Henning, S.J. and Davidson, N.O. (1992) J. Lipid Res. 33, 1843–1856.