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Alterations in Lipid Composition and Fluidity of Liver Plasma Membranes in Copper-Deficient Rats[1] (42743)

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Abstract. In view of the importance of membrane fluidity on cell functions, the influence of phospholipid acyl groups on membrane fluidity, and the changes in lipid metabolism induced by copper (Cu) deficiency, this study was designed to examine the influence of dietary Cu on the lipid composition and fluidity of liver plasma membranes. Male Sprague–Dawley rats were divided into two dietary treatments, namely Cu deficient and Cu adequate. After 8 weeks of treatment, liver plasma membranes were isolated by sucrose density gradient centrifugation. The lipid fluidity of plasma membranes, as assessed by the intramolecular eximer fluorescence of 1,3-di(1-pyrenyl) propane, was significantly depressed by Cu deficiency. In addition, Cu deficiency significantly reduced the content of arachidonic and palmitoleic acids but increased the docosatetraenoic and docosahexaenoic acids of membrane phospholipids. This alteration in unsaturated phospholipid fatty acid composition, especially the large reduction in arachidonic acid, may have contributed to the depressed membrane fluidity. Furthermore, Cu deficiency also markedly altered the fatty acid composition of the triacylglycerols associated with the plasma membranes. Thus, the lipid composition and fluidity of liver plasma membranes are responsive to the animal's Cu status.

The hypercholesterolemic effects of macronutrients such as dietary fats, cholesterol, and proteins have been extensively examined. However, the influence of micronutrients, such as trace elements, on cholesterol and lipoprotein metabolism is less well defined. Among the trace elements, a deficiency of copper (Cu) is capable of inducing hypercholesterolemia in rats (1–5), rabbits (6), and man (7). Studies designed to elucidate the mechanism responsible for the hypercholesterolemia were performed mainly with rats. Copper deficiency was found to exert no influence on the rate of synthesis of hepatic cholesterol (1) and bile acid (2), as well as the excretion of biliary sterol (3). In contrast, kinetic studies indicated marked increases in the size and half-life of the rapidly exchangeable cholesterol pool, consisting of tissues equilibrating rapidly with serum cholesterol, in Cu-deficient rats (4). Furthermore, Cu deficiency prolonged the half-life of free and total cholesterol associated with high density lipoproteins (HDL) (5). Elevations in protein and cholesterol contents of HDL and low density lipoproteins (LDL) and in triglyceride level of LDL were also observed in Cu-deficient rats (8). In addition, the apolipoprotein E (apo E) concentration of HDL (8) and the protein and cholesterol contents of an apo E-rich subclass of HDL were elevated as a result of Cu deficiency (9).

The available data suggest that a receptor-mediated pathway may contribute to the removal of HDL cholesteryl esters via hepatic (10) or steroidogenic (11) tissues. In rats, the clearance of plasma HDL cholesterol *in vivo* does not necessarily result in the uptake of the entire lipoprotein particle, but involves a reversible binding of HDL to cell membrane and a subsequent selective transfer of cholesteryl esters into the cell (12). The importance of the interaction between HDL and cell membrane is emphasized by the recent *in vitro* HDL binding studies which demonstrated alteration in binding when apo E-rich HDL and liver membranes from Cu-deficient rats were used (13).

Alterations in membrane lipid composition such as increases in unsaturation of phospholipid acyl groups (14) and in choles-

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terol content (15) have resulted in increased membrane fluidity. Thus membrane fluidity may be altered by Cu deficiency since changes in hepatic fatty acid composition of phospholipids and triacylglycerols (16, 17) and increased hepatic fatty acid synthesis (1) associated with copper deficiency may affect hepatic membrane lipid composition. In view of the importance of plasma membrane fluidity on cellular activities and functions (18), the present study was performed to determine the influence of copper status on fatty acid profile, lipid composition, and fluidity of hepatic plasma membranes.

Methods. *Animals, diets, and hepatic plasma membrane preparation.* Thirty weanling male Sprague–Dawley rats were randomly allotted into two dietary treatments (copper deficient and adequate). The basal diet (copper deficient) was prepared according to the American Institute of Nutrition specifications (19) except glucose monohydrate was used as the carbohydrate source and no copper supplement was included in the mineral mix. It contained 0.7 mg of Cu/kg of diet as measured by atomic absorption spectrophotometry (20) using certified reference standards (Fisher Scientific Co., Los Angeles, CA). The adequate diet was prepared by adding $CuCO_3$ to the basal diet to attain 8.0 mg of Cu/kg. Diet and distilled-demineralized water were provided *ad libitum.*

Rats were housed individually in suspended stainless-steel wire cages in a laboratory maintained at 22°C with a 12-hr light cycle. After 8 weeks of dietary treatment, rats were fasted for 12 hr and exsanguinated under ether anesthesia. Livers were perfused *in situ,* excised, pooled (three livers per pool), minced, and homogenized immediately in hypotonic buffer (1 m*M* $NaHCO_3$, 0.5 m*M* $CaCl_2$, 1 m*M* phenylmethylsulfonyl fluoride, pH 7.5) using a Polytron homogenizer (3 × 5 sec bursts at setting No. 4). Plasma membranes were then prepared by a modification (21) of the original method of Neville (22) using a discontinuous sucrose gradient system (23). Enrichment of plasma membranes was monitored by assaying for the activity of 5′-nucleotidase (24), a marker enzyme primarily associated with rat hepatic plasma membranes. A 12- to 15-fold enrichment over the crude homogenate was attained. This preparation is subsequently referred to as plasma membranes and should not be considered to have been purified to homogeneity. Plasma membranes were suspended in 0.15 *M* NaCl at 1–2 mg membrane protein/ml and stored frozen at −20°C. Protein concentrations were measured by the method of Lowry *et al.* (25) using bovine albumin as the standard.

Lipid analyses. Total lipids were extracted from membrane preparations by the method of Folch *et al.* (26). Butylated hydroxytoluene (5 mg/100 ml of solvent) was used to prevent peroxidation. The total phospholipid (PL) and cholesterol contents were measured by the methods of Fiske and Subbarow (27) and DeHoff *et al.* (28), respectively. Triglycerides (TG) and PL were separated by thin-layer chromatography using a solvent system of *n*-hexane:diethyl ether:acetic acid:methanol, 90:20:2:3 (v/v) (29). The TG content was estimated by the method of Sardesai and Manning (30). Fatty acids of TG and PL fractions were then converted to fatty acid methyl esters (FAME) by base-catalyzed transesterification (31) with sodium methoxide (32). FAME were measured by a Shimadsu gas chromatograph (Model SA, Kyoto, Japan) equipped with a Supelco Wax 10 wide bore capillary column 30 m × 0.75 mm, injection and column temperature at 300 and 250°C, respectively, and N_2 carrier gas at linear velocity of 50 cm/sec. Peaks were identified by comparison of retention times to those of standard fatty acids (Supelco, Inc. Bellefonte, PA) and peaks were integrated (Spectra Physics SP420 Integrator, Piscataway, NJ).

Intramolecular excimer fluorescence. The dynamic component of membrane fluidity was evaluated by measurements of excimer fluorescence of an intramolecular excimer forming fluorophore, 1,3-di(1-pyrenyl) propane (DPP). DPP has been used to monitor short-range lateral diffusion of pyrene substituents in erythrocyte membranes (33). The method which was modified for rat hepatocyte plasma membranes was used (14).

A 4-μl aliquot of freshly prepared 0.15 m*M* DPP (Molecular Probes, Eugene, OR) was added to 400 μl of Tris-buffered saline (TBS, 5 m*M* Tris, 146 m*M* NaCl, 4 m*M*

KCl, pH 7.4) containing freshly thawed and resuspended membranes equivalent to 400 μg of membrane protein. The mixture was prewarmed to 37°C stirred vigorously for 20 sec and then incubated at 37°C for 1 hr with constant shaking. To each sample, 3 ml of deoxygenated TBS (evacuated and flushed with highly purified N_2 for 30 min) was added. Two milliliters of 20% (wt/vol) Dextran 500 (Pharmacia) was layered under the membrane suspension to prevent the contamination of the suspension by insoluable DPP aggregates. The upper phase containing the membranes was removed after it stood for 10 min. Membranes were pelleted and washed twice with 5 ml of deoxygenated TBS by centrifugation at 40,000*g* for 20 min at 20°C. The washed membrane was suspended in 1.5 ml of deoxygenated TBS and allowed to stand for at least 1 hr at room temperature. Fluorescence emission spectra were recorded at 25°C with a Farrand fluorescence spectrophotometer (Model MK1 corrected spectra system, Valhalla, NY). An excitation wavelength of 345 nm was used. Fluorescence peak intensities of excimer and monomer were measured at 485 and 356 nm, respectively. Sample intensities were corrected for membranes carried through the procedure without DPP and for the probe added to TBS alone.

Results and Discussion. Established indicators of Cu deficiency such as the significantly ($P < 0.001$) depressed weight gain (170 ± 7.0 vs 226 ± 7.1 g), increased heart/body weight percentage ratio (0.61 ± 0.02 vs 0.37 ± 0.01), and depleted hepatic Cu stores (0.88 ± 0.18 vs 3.40 ± 0.20 μg/g wet wt) were observed in rats fed the Cu-deficient as compared to the Cu-adequate diet. Thus, rats fed the deficient diet were indeed Cu deficient.

The purity of membrane preparations was assessed by the marker enzyme 5′-nucleotidase (Table I). A 12- to 15-fold enrichment was observed when the activities of the final membrane preparations were compared with those of crude liver homogenates. The observed activities and degrees of enrichment of the marker enzyme were comparable to values reported in previous studies (14, 34). In the present study, no significant treatment difference was observed for the 5′-nucleotidase activity of the membrane preparations used.

The short-range lateral diffusion in plasma membranes was evaluated by the use of excimer/monomer fluorescence intensity ratio of DPP. The reduction in this ratio observed in membranes of Cu-deficient rats indicates a decreased fluidity (Table I).

In view of the important influence of the cholesterol/phospholipid ratio on the fluidity of lipid membranes (15), the effect of Cu deficiency on the ratio was examined in the present study. However, the cholesterol/phospholipid ratio was not significantly influenced by dietary treatment (Table I). Thus the decrease in membrane fluidity (Table I) is not due to an alteration in this ratio.

The possibility that dietary Cu deficiency may alter the distribution of unsaturated phospholipid acyl group and subsequently decrease the fluidity of the plasma membrane was investigated in the present study

TABLE I. EFFECTS OF COPPER DEFICIENCY ON MARKER ENZYME ACTIVITY, LIPID COMPOSITION, AND FLUIDITY PARAMETER OF LIVER PLASMA MEMBRANES[a,b]

Parameter	Copper adequate	Copper deficient	*P* level
5′-Nucleotidase activity[c]	35.8 ± 4.22	27.1 ± 3.54	NS
Excimer/monomer fluorescence intensity ratio	0.293 ± 0.011	0.241 ± 0.008	<0.01
Cholesterol/phospholipid molar ratio	0.69 ± 0.04	0.70 ± 0.04	NS
Triglyceride/phospholipid molar ratio	0.089 ± 0.023	0.215 ± 0.023	<0.05
Cholesterol (μmole/mg protein)	0.076 ± 0.012	0.060 ± 0.012	NS
Phospholipids (μmole/mg protein)	0.119 ± 0.006	0.090 ± 0.015	NS
Triglycerides (μmole/mg protein)	0.010 ± 0.002	0.029 ± 0.006	<0.05

[a] Means ± SE from five pools of membrane preparation, with the exception of the means for triglycerides and triglyceride/phospholipid ratio where $n = 3$. Each membrane pool was derived from three livers.
[b] Student's *t* test comparisons; NS = nonsignificant.
[c] Micromoles/mg protein/hr.

(Table II). The fatty acid profile of membrane phospholipids, derived from adequate animals, was comparable to that reported previously (14, 34). Small variations in individual fatty acid value between studies may be due to differences in dietary composition, especially the quantity and type of dietary fat used in the various studies. Among the individual fatty acids, marked reductions of arachidonic (20:4, 27%) and palmitoleic (16:1, 21%) acids as well as elevations in docosatetraenoic (22:4, 56%) and docosahexaenoic (22:6, 150%) acids were observed in the Cu-deficient treatment. In terms of their contribution to total fatty composition of membrane phospholipids, the changes in 16:1 and 22:4 were small, but the alterations in arachidonic (5.6% reduction) and docosahexaenoic (3% increase) acids were sizable. Since the present study is the first to address the influence of Cu deficiency on hepatic plasma membrane fatty acid composition, the data will be compared with the study of Balevska *et al.* (35) using hepatic mitochondrial and microsomal membranes. The marked reduction in arachidonic acid and elevation in docosahexaenoic acid were also observed in the different membrane preparations, with similar magnitude of alterations. Most of the changes in fatty acid composition of hepatic plasma membrane phospholipids followed closely the alterations observed in total liver phospholipids in mice (16) and rats (36, 37) as a result of Cu deficiency. These studies demonstrated similar trends in the reductions of 16:1 (16, 37) and 20:4 (16, 36) as well as elevation of 22:6 (16, 36, 37) induced by Cu deficiency as compared to the present study. Thus, changes in the phospholipid fatty acid profile of plasma membranes appeared to reflect the overall alterations in the entire liver. Although the 16:0/16:1 ratio was increased by Cu deficiency, the overall proportion of saturated fatty acids of membrane phospholipids was not altered (Table II). In order to further evaluate the degree of unsaturation, the double-bond index was derived from the sum of product of the fraction of each fatty acid multiplied by the number of double bonds in that acid. When the index was calculated up to and including 20:4, as by Storch and Schachter (14), a significant reduction in this index was observed for membrane phospholipid fatty acids from

TABLE II. EFFECTS OF COPPER DEFICIENCY ON THE FATTY ACID COMPOSITION FROM PHOSPHOLIPIDS AND TRIACYLGLYCEROLS OF HEPATIC PLASMA MEMBRANES[a,b]

Component	Phospholipids		Triacylglycerols	
Fatty acid (% total)	Cu adequate	Cu deficient	Cu adequate	Cu deficient
14:0			11.8 ± 1.9	5.1 ± 0.9*
16:0	25.6 ± 0.6	23.9 ± 0.7	24.9 ± 2.4	25.5 ± 0.5
16:1 *n*-7	1.39 ± 0.11	1.05 ± 0.05*	4.1 ± 0.4	3.1 ± 0.1
18:0	26.8 ± 0.6	24.8 ± 0.2	9.0 ± 0.9	4.2 ± 0.7**
18:1 *n*-9	7.9 ± 0.5	7.8 ± 0.1	15.2 ± 0.9	23.2 ± 1.3**
18:2 *n*-6	10.8 ± 0.2	12.8 ± 0.3	10.9 ± 0.9	23.3 ± 0.6**
18:3 *n*-3	trace	trace	3.1 ± 0.2	0.2 ± 0.1**
20:4 *n*-6	20.3 ± 0.8	14.8 ± 0.8**	2.3 ± 0.5	7.1 ± 0.5**
22:4 *n*-6	0.61 ± 0.02	0.95 ± 0.07**	0.17 ± 0.10	1.25 ± 0.12*
22:5 *n*-6	1.0 ± 0.1	1.5 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
22:5 *n*-3	1.3 ± 0.1	1.4 ± 0.2	2.4 ± 1.8	1.6 ± 0.7
22:6 *n*-3	2.0 ± 0.4	5.0 ± 0.8	5.1 ± 0.5	0.9 ± 0.3**
Unidentified	2.3	6.0	9.8	3.5
16:0/16:1 ratio	18.8 ± 1.4	22.6 ± 0.4*	6.2 ± 0.8	8.3 ± 0.3
18:0/18:1 ratio	3.4 ± 0.2	3.2 ± 0.1	0.60 ± 0.06	0.19 ± 0.03**
Saturated fatty acids (% of total)	52.3 ± 1.2	48.7 ± 0.8	45.7 ± 2.4	34.7 ± 0.6*

[a] Percentage compositions of fatty acids from total membrane phospholipids and triacylglycerols were determined from four different pools of plasma membrane. Each membrane pool was derived from three livers. Means ± SE.
[b] Student's *t* test comparisons: *$P < 0.05$; **$P < 0.01$.

Cu-deficient as compared to adequate rats (0.94 ± 0.03 vs 1.12 ± 0.02, $P < 0.05$). However when the fatty acids up to 22:6 were used in the calculation, the double-bond index was not significantly altered. Thus, a reduction in unsaturation of phospholipid acyl group cannot be firmly established in Cu-deficient liver plasma membranes using the double-bond index as a criterion. Nevertheless, the large reduction of 20:4, a drop of 5.6% of the total phospholipid fatty acid composition, may be a major contributing factor in the reduction in membrane fluidity in Cu-deficient rats. In view of the importance of membrane 20:4 in the peroxidation mechanism as well as being the precursor pool for prostaglandin synthesis, future research should be concentrated on the influence of dietary copper on the mechanisms which control membrane 20:4 metabolism.

Triacylglycerols are not considered to be part of the membrane structure but may be present as small lenses on the membrane surface, or as components of the LDL and HDL bound to membrane lipoprotein receptor or binding sites. Although triacylglycerols have not been implicated as a factor which may influence membrane fluidity, their fatty acid profile was examined because of their association with plasma membrane (Table II). Copper deficiency induced reductions in the proportion of saturated fatty acids (24%) and in 18:0/18:1 ratio (68%) of triacylglycerols. In addition, a marked increase in double-bond index (0.59 ± 0.04 vs 1.02 ± 0.02, $P < 0.01$) was observed in the Cu-deficient as compared to the Cu-adequate group, when the index was calculated up to 20:4, thus providing support for the reductions in the proportion of saturated fatty acid and 18:0/18:1 ratio, which indicated an increase in unsaturation. However, no significant difference in the index was found when calculated up to 22:6. Thus, the index may not be a good indicator of the degree of unsaturation when small amounts of fatty acids greater than 20:4 are included in its calculation. Among the individual triacylglycerol fatty acids, increases in oleic (18:1 *n*-9, 53%), linoleic (18:2 *n*-6, 114%), and arachidonic (20:4 *n*-6, 209%) acids were accompanied by reductions in stearic acid (18:0, 53%) and *n*-3 fatty acids such as linolenic (18:3 *n*-3, 94%) and docosahexaenoic (22:6 *n*-3, 83%). Thus, a preferential incorporation of *n*-9 and *n*-6 fatty acids over *n*-3 fatty acids into membrane-associated triacylglycerols appeared to have resulted from Cu deficiency. It is interesting that most of the alterations are large in magnitude when expressed as percentage of triacylglycerol fatty acids. Similar increases in 18:1 (16), 18:2 (35, 37), and 20:4 (37) as well as a reduction in 18:3 (16) were observed in previous studies examining the triacylglycerol fatty acid profile of whole liver homogenates from Cu-deficient animals. Apart from the marked alterations in fatty acid composition, the triacylglycerol/phospholipid ratio and triacylglycerol value were also increased more than two-fold (Table I). However, the triacylglycerols accounted for only 7–15% of the total lipids of the membrane preparation. This increase in triacylglycerols associated with Cu-deficient membrane may be caused by the drastic changes in triacylglycerol fatty acid composition resulting in more being bound to membrane preparations. Another possibility is that a substantial portion of membrane triacylglycerols may be accounted for by the membrane bound lipoproteins if all lipoprotein receptors or binding sites are occupied. Thus the amount of membrane bound lipoproteins may have contributed to the increased membrane-associated triacylglycerols, since elevated triacylglycerol content of lipoproteins (8) and increased binding affinity of HDL to plasma membranes (13) have been established in Cu-deficient rats. At present, the biological significance of these changes observed in membrane triacylglycerols and their possible contribution to membrane fluidity are not apparent.

The reduction in excimer/monomer fluorescence intensity ratio suggests that lateral diffusion may be impaired in liver plasma membranes of Cu-deficient rats. This may affect the movement of lipoprotein receptors along the membrane surface to be concentrated in the coated pits or alter other membrane events associated with lipoprotein binding and uptake, as well as retroendocytosis of lipoproteins. Any alteration in such processes will change the cellular influx or efflux of cholesterol and may thus contribute to the hypercholesterolemia in Cu-deficient

rats. More importantly, the results of the present study demonstrate that the lipid composition and fluidity of plasma membranes are responsive to the animal's Cu status and suggest that other essential cell functions dependent on optimal membrane fluidity may also be altered.

1. Lei KY. Cholesterol metabolism in copper-deficient rats. Nutr Rep Int **15**:597–605, 1977.
2. Lei KY. Oxidation, excretion and tissue distribution of [26-^{14}C]cholesterol in copper-deficient rats. J Nutr **108**:232–237, 1978.
3. Allen KGD, Klevay LM. Copper deficiency and cholesterol metabolism in the rat. Atherosclerosis **31**:259–271, 1978.
4. Lin IM, Lei KY. Cholesterol kinetic analyses in copper-deficient rats. J Nutr **111**:450–457, 1981.
5. Lei KY, Lin IM. Kinetics of high density lipoprotein cholesterol in copper-deficient rats. Nutr Rep Int **24**:1179–1186, 1981.
6. Hing SAO, Lei KY. Copper deficiency and hyperlipoproteinemia induced by *N,N*-bis-(2-aminoethyl)-1,3-propane diamine (2,3,2-tetramine) in rabbits. Fed Proc **45**:357, 1987.
7. Klevay LM, Inman L, Johnson LK, Lawler M, Makalko JR, Milne DB, Lukaski HC, Bolonchuk W, Sandstead HH. Increased cholesterol in plasma in a young man during experimental copper depletion. Metabolism **33**:1112–1118, 1984.
8. Lei KY, Hassel CA, Allen DK. Alterations in plasma lipid, lipoprotein and apolipoprotein concentrations in copper-deficient rats. J Nutr **113**:2178–2183, 1983.
9. Croswell SC, Lei KY. Effect of copper deficiency on the apolipoprotein E-rich high density lipoproteins in rats. J Nutr **115**:473–482, 1985.
10. Bachorik PS, Franklin FA Jr., Virgil DG, Kwiterovich PO Jr. Reversible high affinity uptake of apo E-free high density lipoproteins in cultured pig hepatocytes. Arteriosclerosis **5**:142–152, 1985.
11. Gwynne JT, Strauss JF III. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocr Rev **3**:299–329, 1982.
12. Glass C, Pittman RC, Civen M, Steinberg D. Uptake of high density lipoprotein-associated apolipoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. J Biol Chem **260**:744–750, 1985.
13. Hassel CA, Carr TP, Marchello JA, Lei KY. Apolipoprotein E-rich HDL binding to liver plasma membranes in copper-deficient rats. Proc Soc Exp Biol Med **187**:296–308, 1988.
14. Storch J, Schachter D. Dietary induction of acyl chain desaturases alters the lipid composition and fluidity of rat hepatocyte plasma membranes. Biochemistry **23**:1165–1170, 1984.
15. Ladbrooke BD, Chapman D. Thermal analysis of lipids, proteins and biological membranes. Chem Phys Lipids **3**:304–356, 1969.
16. Cunnane SC, McAdoo KR, Prohaska JR. Lipid and fatty acid composition of organs from copper-deficient mice. J Nutr **116**:1248–1256, 1986.
17. Singh NP, Medeiros DM. Effect of copper deficiency and sodium intake upon liver lipid and mineral composition in the rat. Biol Trace Elem Res **6**:423–429, 1984.
18. Spector AA, Yorek MA. Membrane lipid composition and cellular function. J Lipid Res **26**:1015–1035, 1985.
19. American Institute of Nutrition. Report of the AIN Ad Hoc Committee on standards for nutritional studies. J Nutr **107**:1340–1348, 1977.
20. Instruction Manual, Model 180-70 Polarized Zeeman Atomic Absorption Spectrophotometer. Nissei Sangyo America, Hitachi Ltd., Mountain View, CA., 1980.
21. Ray TK. A modified method for the isolation of the plasma membrane from rat liver. Biochim Biophys Acta **196**:1–9, 1970.
22. Neville DM Jr. The isolation of a cell membrane fraction from rat liver. J Biophys Biochem Cytol **8**:413–422, 1960.
23. Song CS, Rubin W, Rifkind AB, Kappas A. Plasma membranes of the rat liver: Isolation and enzymatic characterization of a fraction rich in bile canaliculi. J Cell Biol **41**:124–132, 1969.
24. Widnell CC, Unkeless JC. Partial purification of a lipoprotein with 5'-nucleotidase activity from membranes of rat liver cells. Biochemistry **61**:1050–1057, 1968.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem **193**:265–275, 1951.
26. Folch J, Lees M, Sloan-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**:497–509, 1957.
27. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem **66**:375–400, 1925.
28. DeHoff JL, Davidson LM, Kritchevsky D. An enzymatic assay for determining free and total cholesterol in tissue. Clin Chem **24**:433–435, 1978.
29. Brown JL, Johnston JM. Radioassay of lipid components separated by thin-layer chromatography. J Lipid Res **3**:480–481, 1962.
30. Sardesai VM, Manning JA. The determination of triglycerides in plasma and tissues. Clin Chim **14**:156, 1968.
31. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res **5**:600–608, 1960.
32. Tahin QS, Blum M, Carafoli E. The fatty acid composition of subcellular membranes of rat liver, heart,

and brain: Diet induced modifications. Eur J Biochem **121**:5–13, 1981.
33. Zachariasse KA, Vaz WLC, Sotomayor C, Kuhnle W. Investigation of human erythrocyte ghost membranes with intramolecular excimer probes. Biochim Biophys Acta **688**:323–332, 1982.
34. Emmelot P, Bos CJ, Van Hoeven RP, Van Blitterswijk WJ. Isolation of plasma membranes from rat and mouse livers and hepatomas. In: Colowick SP, Kaplan NO, Eds. Methods in Enzymology. New York; Academic Press, Vol. 31:p. 75–90, 1974.
35. Balevska PS, Russanov EM, Stanchev PL. Copper deficiency-induced changes in the fatty acid composition of mitochondrial and microsomal membranes of rat liver. Biol Trace Elem Res **8**:211–218, 1985.
36. Ovecka GD, Miller G, Medeiros DM. Fatty acid composition of selected tissues of copper deficient rats. Fed Proc **46**:910, 1987; and personal communication.
37. Cunnane SC, Horrobin DF, Manku MS. Contrasting effects of low or high copper intake on rat tissue lipid essential fatty acid composition. Ann Nutr Metab **29**:103–110, 1985.

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